NATIONAL CANCER INSTITUTE

ANNUAL REPORT

July 1, 1975 through June 30, 1976

Part II-B

DIVISION OF CANCER BIOLOGY AND DIAGNOSIS

SUMMARY

Tumor Immunology Contract Program
July 1, 1975 through June 30, 1976

An immunology program has been designed which has as its goals 1) the use of immunologic techniques to detect agents etiologic for cancer, 2) the prevention of cancer by immunologic manipulations, 3) the use of immunologic techniques to improve the diagnosis of cancer, and 4) the manipulation of the immune system of patients with established cancer so that increased survival is achieved.

The contract mechanism has been used to achieve these goals through the support of relevant investigations. Thus far, implementation has been directed toward 1) studies of fundamental problems (designated immunobiology), the solutions to which are required for achieving the four goals, 2) immunodiagnostics, and 3) immunotherapy. All projects have been assigned to one of these three categories although many projects include research in more than one category.

A. IMMUNOBIOLOGY

1. Identification and characterization of surface membrane components of tumor cells and cells of the immune system. Projects in this area include studies of (1) tumor associated antigens and corresponding antibodies (43921, 53942, 43858); (2) biochemical, serologic and immunogenetic characterization of normal and tumor cell surface antigens (43920, 43986, 43925, 43985, 44009, 53876); (3) organization and dynamics of cell surface membrane components relevant to tumor immunology (33879, 43922, 43987, 44008, 44000).

2. Investigations of reactivity of hosts to tumors and identification of cells involved. Projects in this area include studies of (1) differentiation of immune cells and determination of roles of different components in immunologic reactions to tumors (23889, 33854, 33859, 33866, 33868, 33870, 43868, 53926, 53957, 63977, 63973, 23890, 53868, 43926, 43927, 43970, 43929, 43932, 43971, 43994, 43997, 43998); (2) antigen binding by T-cells and transplantable T cell tumors (43923, 43999, 63993, 53878, 43924); (3) mechanisms for tumor cell destruction and means of escape from these (63978, 63982, 63992, 43930, 43931, 44001; (4) tumor neo-vasculazation (43942) and (5) genetic control of immune responses in relation to cancer (43934, 43972).

3. Development, maintenance and distribution of special experimental animals' tumors and cell lines and development or improvement of techniques needed in tumor immunology. Projects in this area include (1) maintenance and distribution of mouse tumors and cell lines (23886); (2) development and distribution of H-2 recombinant strains of mice (43941, 43935, 43988); (3) development of improved techniques for in vitro immunologic studies (23883, 63979, 53881, 63989, 53958); and (4) development of a bulk electrophoretic method for separation of sub-populations of lymphocytes (43928).

B. IMMUNODIAGNOSIS

1. Evaluate use of carcinoembryonic and other fetal antigens in the diagnosis of human cancer. Projects in this area include (1) development of more sensitive assays (23890); (2) attempts to better understand the chemical composition and structurally characterize fetal antigens (33264, 43277, 43989); and (3) clinical studies of usefulness in diagnosis or monitoring of patients with malignancy (33858, 33875, 43887, 43888, 53934).

2. Search for new tumor associated antigens and tissue antigens in human cancer which may be useful in immunodiagnosis. Projects in this area include (1) animal models for circulating tumor associated antigens (43891); (2) investigation of other extraction procedures for glycoproteins from gastrontestinal cancer (43859); (3) extraction of antigens and immunization of heterologous species to develop new potentially useful diagnostic reagents (43860, 43889, 43958, 43890); (4) evaluation of tissue antigens for diagnosis (53896).

3. Evaluate diagnostic usefulness of serologic assays and of humoral immune response to tumor associated antigens. Projects in this area include (1) develop animal models and more sensitive techniques for early detection of antibodies to tumor associated antigens (43882); (2) identification of antibodies to autologous human cancer (43881, 43953); (3) serum collections for evaluation of serum diagnostic tests (23879, 33914, 33915); and (4) detection of circulating antigen-antibody complexes (53893, 63990, 53952).

4. Evaluate diagnostic usefulness of cell-mediated immune response to tumor associated antigens. Projects in this area include (1) improved methodology of assays for cell-mediated immunity (43965, 43893); (2) improved procedures for preparation of effector lymphocytes and target cells (43854, 43964, 43885, 43883, 53900); (3) isolation of antigens active in in vivo and in vitro assays of cell-mediated immunity (53899, 53954, 53955); (4) evaluate usefulness of assays for depression in cell-mediated immunity in cancer patients (43856); (5) evaluation of diagnostic potential of antibody dependent cell-mediated cytotoxicity (53894); and (6) evaluation of usefulness of assays for specific cell-mediated immunity in cancer patients (53901, 53956).

C. IMMUNOTHERAPY

1. Clinical research. Since the initiation of the tumor immunology contracting program, 16 clinical trials of immunotherapy have been initiated, and 8 of these were started in fiscal 1975. Early trials emphasized the study of tumors in which the immunotherapeutic approach appeared to be most promising including melanoma and acute myelogenous leukemia (23887, 23885, 33373, 33888, 43878, 43852, 43879). Results from these and other early trials have led the Committee on Cancer Immunotherapy to recommend that additional trials be undertaken in patients with other tumors such as lung and breast cancer (53939, 53941, 53933, 53873, 53940, 53875). In addition, resource contracts have been initiated with two medical centers which will permit phase I evaluation of immunotherapeutic agents (53874 and 53970). Although immunotherapy as presently administered does not produce dramatic cures or remissions

of disease, early evidence in melanoma, acute myelogenous leukemia, and lung cancer indicates that this approach to cancer therapy is becoming established as a fourth modality in addition to surgery, radiation, and chemotherapy. Very recent results in stage I lung cancer suggest that the introduction of BCG into the pleural space after removal of the primary lung tumor will significantly prolong the disease free interval (53940). This is particularly impressive since other therapeutic approaches have not significantly influenced the course of this disease.

2. Preclinical research. There are many unresolved questions regarding immunologic mechanisms relevant to immunotherapy and as these mechanisms are identified by research in the immunobiology of cancer, it is necessary to find ways to manipulate these mechanisms in an optimal manner. The program is promoting work to find answers to these broad and basic questions through contracts utilizing animal models (43887, 33891, 23221, 43874, 43951). In addition, other contracts are evaluating new methods for immunotherapy and evaluating the mechanisms of action of the immunotherapeutic agents (43949, 53936, 53937, 53869, 63988, 53872, 02208, 43874, 43946, 43948, 43873, 43967, 53885).

Several contracts serve as resources for special needs in the immunotherapy area (43954, 53870).

CONTRACT RESEARCH SUMMARY

Title: Genetic Control of Immune Responses in Relation to Cancer

Principal Investigator: Frank Lilly, Ph.D.
Name/Address Performing Organization: Albert Einstein College of Medicine
Bronx, New York 10461

Contract Number: NO1-CB-43934
Starting Date: 5/16/74 Expiration Date: 5/13/76

Goal: To identify single genes and/or polygenic systems involved in immune responses and to determine their relationship to tumor immunity.

Approach: The contractor will study various strains of mice for responsiveness to H-2 D.2 antigen. Congenic strains will be used to identify H-2 linked and H-2 independent genes involved in the response.

Progress: Progress is being made in determining why B10 A(5R) mice do not make cytotoxic anti-H-2D^b antibodies when immunized with B-10 normal spleen cells.

Significance for Cancer Research (NCP Objective 2 Approach 3)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY 76 Funds: $72,405

CONTRACT RESEARCH SUMMARY

Title: Organization and Dynamics of Cell Surface Membrane Components

Principal Investigator: Dr. Richard A. Rifkind
Name/Address College of Physicians and Surgeons
Performing of Columbia University
Organization: New York, New York 10032

Contract Number: N01-CB-44008
Starting Date: 5/1/74 Expiration Date: 4/30/76

Goal: To increase understanding of the organization and dynamics of macromolecular components of normal and malignant cell surfaces.

Approach: The contractor will study cell surface properties associated with viral transformation of erythroid precursor cells and the effects of such alterations of surface properties on differentiation and proliferation.

Progress: Studies have been done to detect changes in cell surface properties which accompany and may be critical to drug-induced erythroid differentiation in murine erythroleukemia cells (MELC). Efforts have been focused on development of three tools for further exploration of the implications of cell surface alterations for the process of drug-induced leukemic cell alteration. New chemical agents with defined properties which induce MELC have been developed, as have variant MELC lines which display different patterns of sensitivity to induction by a panel of inducing chemicals. Additional techniques to study surface properties and better define surface events during differentiation have been applied, especially freeze-etching and testing pharmacologic agents with known cell membrane activities.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $51,094

CONTRACT RESEARCH SUMMARY

Title: Production and Distribution of H-2 Recombinant or Mutant Congenic Strains of Mice

Principal Investigator: Dr. Jack H. Stimpfling
Name/Address Columbus Hospital
Performing Great Falls, Montana 59401
Organization:

Contract Number: NO1-CB-43935
Starting Date: 6/1/74 Expiration Date: 5/31/76

Goal: To facilitate the development of suitable production and distribution centers to provide H-2 recombinant or mutant congenic mice in adequate numbers for immunogenetic studies.

Approach: The contractor will produce and distribute to other qualified investigators up to 100 mice per month of at least 5 different H-2 recombinant strains of mice.

Progress: More than 900 congenic recombinant mice have been provided to 18 investigators to date. A notice of availability of 21 recombinant lines has been sent to Mouse Newsletter and to 100 investigators.

Significance for Cancer Research (NCP Objective 2 Approach 3)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $20,951

CONTRACT RESEARCH SUMMARY

Title: Subpopulation Specific Antisera

Principal Investigator: Dr. Patricia Byefield
Name/Address Harbor General Hospital
Performing Torrance, California 90502
Organization:

Contract Number: NO1-CB-63989
Starting Date: 9/01/75 Expiration Date: 8/31/76

Goal: To create reagents useful for distinguishing and separating lymphocyte subpopulations with similar morphology and different function.

Approach: The contractor will try to produce sub-population specific antisera by immunizing chickens and rabbits with spleen tissue from horn sharks.

Progress: New Contract

Significance for Cancer Research (NCP Objective 4 Approach 1)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $25,925

CONTRACT RESEARCH SUMMARY

Title: Isolation, Characterization and Immunochemistry of Tumor Angiogenesis Factor (TAF)

Principal Investigator: Bert L. Valle
Name/Address Harvard Medical School
Performing Boston, Massachusetts
Organization:

Contract Number: NO1-CB-43942
Starting Date: 3/1/74 Expiration Date: 2/28/76

Goal: To purify and characterize TAF and produce the corresponding antibody.

Approach: TAF will be purified to homogeneity and characterized as to chemical composition. Throughout purification in vivo bioassays will be carried out to confirm its specific activity, i.e. ability to induce neovascularization.

Progress: Continuous cultures of tumor cells have been grown to produce active TAF. A transformed mouse line KNIH fibrosarcoma was discovered to have superior growth characteristics to the original Walker sarcoma line used. TAF activity was found to be stable in phosphate and tris buffers at neutral PH.

Significance for Cancer Research (NCP Objective 4 Approach 2)

If neo-vascularization could be prevented by antibody against TAF, tumor growth might be prevented.

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date: October 5, 1973
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $175,656

CONTRACT RESEARCH SUMMARY

Title: Specificity of Antigen-Binding Receptors on T-Cells

Principal Investigator: V. E. Olavi-Makela
Name/Address Performing Organization: Helsinki University
Helsinki, Finland

Contract Number: NO1-CB-43923
Starting Date: 6/1/74 Expiration Date: 5/31/76

Goal: To investigate the properties of antigen-binding receptors on antigen-activated T lymphocytes.

Approach: The contractor will study the fine specificity of various manifestations of acquired immunity and will also undertake studies on genetically controlled differences in fine specificity of anti-NP antibodies.

Progress: The contractor demonstrated in SDK rats that fine-specificity of anti-ABA-Tyr immunity is different in MIF production and in humoral antibodies. A Mendelian gene locus has been characterized in the mouse which is linked to the Ig heavy chain allotype. This locus controls the fine-specificity of anti-ABA-HOP and anti-ABA-Tyr antibodies. At least three alleles were found. A polymorphism was discovered in the new antibody anti-SULF-HOP and may be caused by a new V gene.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $28,800

CONTRACT RESEARCH SUMMARY

Title: Cell-Mediated Immunity to Epstein-Barr Virus

Principal Investigator: Dr. David Stevens
Name/Address Performing Organization: Institute for Medical Research of Santa Clara County
751 South Bascom Avenue
San Jose, California 95128

Contract Number: NO1-CB-53957
Starting Date: 6/16/75 Expiration Date: 7/15/77

Goal: Gaining information of prognostic and possibly etiologic value from studies performed on cancer patients.

Approach: Establishment of assays of cell-mediated immunity to Epstein-Barr Virus antigens, purification of these antigens and determination of which antigens are especially potent in stimulating cell-medicated immunity. Studies will be made of correlations between results of these assays and the clinical course of patients with lymphoma or infectious mononucleosis.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $26,701 (for entire 2 year contract)

CONTRACT RESEARCH SUMMARY

Title: Production and Distribution of H-2 Recombinant or Mutant Congenic Strains of Mice

Principal Investigator: Marianna Cherry, Ph.D.
Name/Address Jackson Laboratory
Performing Bar Harbor, Maine 04609

Contract Number: NO1-CB-43988
Starting Date: 6/1/74 Expiration Date: 5/31/76

Goal: To facilitate the development of suitable production and distribution centers to provide H-2 recombinant or mutant congenic mice in adequate numbers for immunogenetic studies.

Approach: The contractor will make available for distribution mice of 8 H-2 recombinant congenic strains, 3 H-2 mutant congenic strains and 3 inbred partners of the congenic strains. Genetic testing will be carried out within lines and between appropriate lines to screen for recent mutations.

Progress: After suitable genetic testing and quarantine procedures, 7 H-2 recombinant congenic strains, 3 H-2 mutant congenic strains and 3 inbred partner strains have been transferred into the Jackson Laboratory production facility. Availability of these mice for distribution to investigators was announced in Mouse Newsletter.

Significance for Cancer Research (NCP Objective 2 Approach 3)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $22,938

CONTRACT RESEARCH SUMMARY

Title: Organization and Dynamics of Cell Surface Membrane Components

Principal Investigator: Dr. Michael Edidin
Name/Address Johns Hopkins University
Performing Baltimore, Maryland 21218
Organization:

Contract Number: NO1-CB-43922
Starting Date: 5/1/74 Expiration Date: 4/30/76

Goal: To increase understanding of the organization and dynamics of macromolecular components of normal and malignant cell surfaces.

Approach: The contractor will fuse mouse and human cells of normal and transformed lines and study antigen mobility.

Progress: The surface diffusion of membrane proteins, and the possible of H-2 genotype and phenotype on cell adhesions were studied. Studies on the levels of potassium ion as moderators of surface antigen mobility were done. In fusions of transformed x transformed cells, 10-fold increased external potassium enhances antigen mobility while 10- or 100-fold decreased potassium concentration arrests antigen diffusion. The potassium ion effect is seen only after cells are incubated in high or low potassium balanced salt solutions prior to fusion. In studies of correlation between cell adhesiveness with H-2 halotype, it was found that H-2 halotype effects can be demonstrated when adhesion of single cells to monolayers is examined with fibroblasts derived from embryos of congenic resistant mouse strains.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $58,657

CONTRACT RESEARCH SUMMARY

Title: Mechanisms for Cell-Mediated Destruction of Tumor Cells

Principal Investigator: Dr. Christopher S. Henney
Name/Address Johns Hopkins University
Performing Organization: Baltimore, Maryland 21218

Contract Number: NO1-CB-43932
Starting Date: 5/1/74 Expiration Date: 4/30/76

Goal: To study early cell surface changes associated with destruction of tumor target cells.

Approach: The contractor will evaluate the protein synthetic requirements for cytolysis, studying the effects of various inhibitors and the kinetics of the efflux of material of various molecular sizes from damaged target cells.

Progress: Studies have concentrated on destruction of antibody-coated human liver cells (Chang cells) by effector cells from human peripheral blood. Conclusions made on the basis of studying kinetics of target cell destruction are that lysis results from single events of collision between an effector and a target cell and that the effector (K) cell operational in the system has limited lytic capacity and is contrasted by the effector T cells whose lytic activity is not compromised by interaction with homologous target cells. It appears that functional inactivation of the K cell may be due to occupation of the cell's Fc receptor by immune complexes. An estimation of frequency of K cells in human peripheral blood was made and was close to a morphologic evaluation of the incidence of "null" cells in the same populations. Studies also showed that concentrations of drugs required to inhibit K cell lysis were similar to those required to suppress effector T cells.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $53,380

CONTRACT RESEARCH SUMMARY

Title: Biochemistry of Normal and Tumor Cell Surface Antigens

Principal Investigator: Dr. Saul Roseman
Name/Address Johns Hopkins University
Performing Baltimore, Maryland 21218
Organization

Contract Number: NO1-CB-43985
Starting Date: 5/16/74 Expiration Date: 5/15/76

Goal: To isolate, purify and chemically characterize cell surface antigens.

Approach: The contractor will isolate cell surface components involved in intracellular adhesion, prepare antibodies against these specific molecules and use such antibodies in studies of effects on the adhesive process per se, time of appearance of antigens during embryonic development and relationship with other cell surface components.

Progress: Scanning and transmission E.M. were used to study morphological changes in cell-cell adhesion and with adhesion of cells to sephadex beads modified with analogues of cell surface carbohydrates. Intercellular adhesion kinetics were examined in detail and it was demonstrated that formation of stable aggregates involved two steps. The first step is reversible and independent of metabolic energy. The second is irreversible and dependent of metabolic energy. Continuing studies include thos on interactions between cells and insoluble analogues of cell surface carbohydrates and those to identify lipid components which stimulate or inhibit intercellular adhesion.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $73,228

CONTRACT RESEARCH SUMMARY

Title: The Role of T-lymphocytes in Tumor-Associated Immunity

Principal Investigator: Dr. Birger Andersson
Name/Address Karolinska Institute
Performing S-104 01, Stockholm 60, Sweden

Contract Number: NO1-CB-33866
Starting Date: 3/25/74 Expiration Date: 3/24/76

Goal: To evaluate the role of B-cell immunity in <u>in vivo</u> tumor rejection using specially prepared animals.

Approach: Tumor rejection capacity will be evaluated by innoculation of titrated tumor doses and measurement of antibody production. At least three well-defined tumors will be used in these assays.

Evaluation of T-cell effects will be made utilizing at least two different methods of T-cell reconstitution.

In utilizing bone marrow for B-cell reconstitution, effort will be made to evaluate or eliminate the presence of contaminating T-cells.

Progress: Thymectomized mice reconstituted with mature B-cells, T-cells, or B and T cells have been used in studies of the effector phase of the immune response to Moloney Leukemia Virus induced tumors in mice. Cell-mediated antibody dependent cytotoxicity was shown to be induced by tumor specific IgM antibody as well as to IgG antibody. Model systems with chemically well-defined antigens such as BSA were also studied.

Significance for Cancer Research (NCP Objective $\frac{3}{6}$ Approach $\frac{2 \& 3}{1 \& 4}$)
Understanding the role of types and relative numbers of T and B cells in immune reactions is basic to manipulation of immune defense against cancer.

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunobiology Coordinating Committee
FY76 Funds: $20,000

CONTRACT RESEARCH SUMMARY

Title: Cell Synergy in Cell Bound Immune Reactions

Principal Investigator: Dr. Henric Blomgren
Name/Address Karolinska Institute
Performing S-104 01, Stockholm 60, Sweden
Organization:

Contract Number: NO1-CB-33868
Starting Date: 3/25/74 Expiration Date: 3/24/76

Goal: 1) To evaluate synergism between thymus cells and lymph node cells in graft versus host and tumor rejection reactions, and 2) to evaluate T-cell tolerance after passage through allogeneic hosts.

Approach: Project 1 will evaluate the interrelations between thymocytes and lymph node cells in several situations as follows: a) synergistically in graft versus host responses, b) antagonistically in graft versus host responses, c) synergistic effects on the rejection of allogeneic tumors, and d) synergy in reactions between thymocytes and lymph node cells in the mixed lymphocyte reaction (MLR). In particular, the kinetics of regenerating thymus cells, their circulating characteristics, and their PHA responsiveness will be evaluated.

Project 2 will be a study of the development of immunologic tolerance in T-cell populations passaged through allogeneic hosts. A second part of this project will be evaluation of the specific tolerance that can be seen in the donor bone marrow that is used to reconstitute lethally irradiated animals shortly after irradiation. In particular, the duration of tolerance will be evaluated and the question of whether bone marrow from such a chimera can be used to protect lethally irradiated mice syngeneic with the marrow will be examined.

Progress: Investigators on this project are making excellent progress with two related problems, one involving a soluble mitogen released by peripheral lymphocytes when stimulated by a phytomitogen and the other involving dissociation of humoral and cellular responses.

Significance for Cancer Research (NCP Objective $\frac{3}{6}$ Approach $\frac{3 \& 4}{1 \& 4}$)

Understanding the role of types and relative numbers of T and B cells in immune reactions, including tolerance, is basic to manipulation of immune defense against cancer.

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $30,000

CONTRACT RESEARCH SUMMARY

Title: Human Tumor-Associated Antigens and Corresponding Antibodies

Principal Investigator: Dr. Eva Klein
Name/Address Karolinska Institute
Performing Stockholm, Sweden
Organization:

Contract Number: NO1-CB-43921
Starting Date: 6/1/74 Expiration Date: 5/31/76

Goal: To learn more about antigens and antibodies related to malignant transformation, for potential applicability to development of diagnostic tests and therapeutic approaches for cancer.

Approach: The contractor will attempt to identify and characterize tumor associated antigens and antibodies in sarcoma, carcinoma and glioma patients. The immunoglobulin coating of tumor cells will also be studied.

Progress: This contract is concerned with the search for tumor associated antigens and antibodies in sarcoma, carcinoma and glioma patients. Stimulation of blood lymphocytes by in vitro exposure to autologous biopsy cells has been observed in some patients and the stimulation doesn't occur if autologous serum is present during the period of interaction or if stimulator cells are preincubated in autologous serum. Evidence for serum cross reactivity between patients with the same tumor type has been uncovered and is being followed. Further characterization of lymphocytes stimulated by autologous tumor biopsies and those infiltrating the tumors will proceed.

Significance for Cancer Research (NCP Objective 3 Approach 4)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $59,760

CONTRACT RESEARCH SUMMARY

Title: Effector Mechanisms in Tumor-Associated Immune Reactions

Principal Investigator: Dr. Eva Klein
Name/Address Karolinska Institute
Performing S-104 01 Stockholm, Sweden

Contract Number: N01-CB-33870
Starting Date: 6/25/73 Expiration Date: 3/24/76

Goal: To study the mechanisms of B-cell action in tumor systems.

Approach: At least 6 different model systems shall be used for the following studies: 1) the role of antibody effect in cytotoxicity, 2) Burkitt lymphoma and mouse lymphoma in nude mice, 3) human B-cells in relation to Burkitt cell in culture, 4) human glioma patients' lymphocytes in relation to glioma cell lines, 5) Marek's disease, and 6) evaluation of human chronic lymphatic leukemia cells for capacity to act as cytotoxic cells.

Progress: Studies are being pursued on natural killer activity of syngeneic or semi-syngeneic mouse spleen cells against in vitro maintained Moloney lymphoma lines, and it has been shown that there is a strong genetic component to the reaction. Other studies involve cross-reactivity of methylcholanthrene-induced tumors, immunoglobulin coating of human lymphoma cells in vivo, and effector and target studies on established lymphoblastoid cell lines.

Significance for Cancer Research (NCP Objective $\frac{3}{6}$ Approach $\frac{4}{1}$)
$\frac{6}{4}$

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $43,000

CONTRACT RESEARCH SUMMARY

Title: Roles of Purified Cells and Antibodies in Cell-mediated Lysis

Principal Investigator: Dr. Hans Wigzell
Name/Address Karolinska Institute
Performing S-104 01, Stockholm 60, Sweden

Contract Number: NO1-CB-33859
Starting Date: 3/25/74 Expiration Date: 3/24/76

Goal: To evaluate several different proposed methodologies for the purpose of achieving high concentrations of T cells and B cells independently of each other.

Approach: The following methodologies will be thoroughly evaluated: 1) the use of antigen coated bead columns to cause adherence of B cells with the subsequent use of dextranase to dissolve the beads, 2) selective cytolysis of T cells with specific anti-T cell antibody, and 3) the evaluation of monolayers of appropriate target cells to achieve adherence of specifically sensitized lymphocytes.

For monitoring, at least three target systems will be used, chick red blood cells, tumor cells (human or mouse), and histoincompatible fibroblasts or tumor cells.

Progress: Progress on improving techniques for purifying subpopulations of lymphoid cells has been excellent, and a variety of techniques have been used effectively, namely (1) rosette sedimentation, (2) selective cytotoxicity, (3) specific adsorption of cells to monolayers and (4) affinity chromatography on beads.

Significance for Cancer Research (NCP Objective 3, 6 Approach 3 & 4)

The ability to work with purified populations of immunocompetent cells is basic to understanding and manipulating immune control of cancer.

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $58,000

CONTRACT RESEARCH SUMMARY

Title: Role of the Macrophage in Tumor Resistance

Principal Investigator: Dr. D. S. Nelson
Name/Address Kolling Institute of Medical Research
Performing Royal North Shore Hospital
Organization: St. Leonards N.S.W., Australia

Contract Number: NO1-CB-63973
Starting Date: 7/16/75 Expiration Date: 10/31/76

Goal: Elucidation of the role of macrophages in the tumor specific and general immune response of tumor bearing hosts.

Approach: The contractor will study the role of macrophages as principal effector cells or amplifying effector cells in the expression of antitumor immunity. He will also study cellular interactions in the production of macrophage cytotoxicity and inhibition of immunity, and also the mode of action of substances which non-specifically increase resistance to tumors and also increase macrophage activity in the induction of tumor immunity.

Progress: New Contract

Significance for Cancer Research (NCP Objective 2 Approach 1)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $74,184

CONTRACT RESEARCH SUMMARY

Title: Identification, Quantitation and Characterization of Lymphocytes and Macrophages

Principal Investigator: Dr. Nicholas Catsimpoolas
Name/Address Massachusetts Institute of Technology
Performing Cambridge, Massachusetts 02139
Organization:

Contract Number: NO1-CB-43928
Starting Date: 6/1/64 Expiration Date: 5/31/76

Goal: To develop improved methods of quantitatively recovering and identifying, separating, quantitating and characterizing sub-populations of lymphocytes.

Approach: The contractor will develop a bulk electrophoretic method for separation of specific sub-populations of surface antigen receptor bearing lymphocytes.

Progress: By using a preparative density gradient electrophoresis method devised by the contractor, lymphocytes were separated into subpopulations. Mouse spleen lymphocytes were separated into seven electrophoretically distinct peaks with complete separation of T and B cells. Differences in surface charge between normal mouse thymus and spleen lymphocytes and nude mouse spleen lymphocytes were demonstrated. A bimodal electrophoretic distribution of human peripheral lymphocytes can be converted to unimodal by incubating cells in 40% autologous plasma, suggesting alteration of lymphocyte surface charge upon incubation. Differential ^{51}Cr uptake was observed for high and low mobility cells, finding which has importance in evaluation of cytotoxic assays where ^{51}Cr labelled human lymphocytes are used as targets. An improved method of preparative velocity sedimentation at 1 g was developed for separation of lymphocytes. Also developed were a new computerized transient velocity sedimentation and a computerized electrophoresis method for repetitive optical monitoring of migrating cell populations.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $111,441

CONTRACT RESEARCH SUMMARY

Title: Serologic and Immunogenetic Investigation of Cell Surface Antigens

Principal Investigator: Ian McKenzie, M.D., Ph.D.
Name/Address Melbourne University
Performing Victoria, Australia
Organization:

Contract Number: NO1-CB-43925
Starting Date: 6/1/74 Expiration Date: 5/31/76

Goal: To develop new test systems and serologic reagents for detection and characterization of cell surface antigens relevant to tumor immunobiology.

Approach: The contractor will study Ly-4.2 in normal and tumor tissues and will determine the correlation of Ly-4.2 with other B cell markers. Studies of other Ly antigens will also be undertaken.

Progress: Particular attention has been paid to the specificities Ly 4.2 and Ly 4.1; Ly-5.1 and Ly 5.2; and Ly 6.2 and Ly 7.2. Antisera has been produced to Ly-1, Ly-2, and Ly-3 specificities and to several new "Ly" specificities. Studies have also been done on the "Ia" system of specificities which are present predominantly on B cells.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $48,315

CONTRACT RESEARCH SUMMARY

Title: Investigations of the Nature and Function of Immune-Related Cells In Tumor Masses

Principal Investigator: Dr. John W. Kreider
Name/Address Performing Organization: Milton S. Hershey Medical Center
Hershey, Pennsylvania

Contract Number: NO1-CB-43931
Starting Date: 5/1/74 Expiration Date: 4/30/76

Goal: To provide information about the numbers of immune-related cells in tumor masses, their identification, function in the tumor mass and factors influencing their movement.

Approach: The contractor will quantitate and identify immunocytes in tumor mononuclear infiltrates, relating the ratiol of lymphocytes of all types to tumor cells with in situ rates of death and proliferation. Immunocyte functional activity will be assessed in vivo and in vitro.

Progress: Quantitative analysis of tumor mononuclear infiltrate (TMI) of progressing and regressing Moloney sarcomas has been completed. It has been concluded that there were small but significant differences in the numbers of proportions of the various types of leukocytes infiltrating progressing or regressing Moloney sarcomas. The essential distinction may reside in the functional activity of a relatively small number of leukocytes. Identification and quantitation of the TMI of progressing and regressing line 1 tumors is in progress.

Significance for Cancer Research (NCP Objective 4 Approach 1)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $69,118

CONTRACT RESEARCH SUMMARY

Title: Selective Stimulation or Suppression of Humoral or Cellular Immunologic Responses

Principal Investigator: Dr. Yves Borel
Name/Address New England Medical Center
Performing 171 Harrison Avenue
Organization: Boston, Massachusetts 02111

Contract Number: NO1-CB-43970
Starting Date: 5/1/74 Expiration Date: 4/30/76

Goal: To facilitate studies of the mechanisms involved in immunologic responses to tumor cells by separating components of the response.

Approach: The contractor will study the interaction of lymphoid cells, their membrane receptors, and their specific antigenic determinants during the induction and maintenance of immunologic tolerance.

Progress: This contract year's studies have shown that receptor blockade and hapten specific carrier-determined tolerance are directly related. It appears that receptor blockade is due to a higher avidity of binding of tolerogenic compounds to the receptor of the antigen binding cell. There are results of some preliminary studies suggesting that the "tolerant cell" suppresses immunity in other cells. With specific antibody to the hapten and complement the tolerant cell can be killed and immunity is subsequently restored, suggesting the function of the cells with blocked receptors is to mediate tolerance. It appears that the tolerant cell (antigen-binding cell with tolerogen on its surface) is a T cell.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $70,459

CONTRACT RESEARCH SUMMARY

Title: Organization and Dynamics of Cell Surface Membrane Components

Principal Investigator: Donald F. H. Wallach, M.D.
Name/Address Tufts - New England Medical Center
Performing 171 Harrison Avenue
Organization: Boston, Massachusetts 02111

Contract Number: NO1-CB-44000
Starting Date: 6/1/74 Expiration Date: 5/31/76

Goal: To increase understanding of the organization and dynamics of macromolecular components of normal and malignant cell surfaces.

Approach: The contractor will undertake fractionation and characterization of plasma membranes of normal and neoplastic lymphocytes, using normal and SV40 transformed lymphoid cells as a model. Membrane dynamics will be studied and an attempt will be made to establish the dynamic topology of normal and SV-40 transformed lymphocytes.

Progress: A system of sequential isopynic ultracentrifugation in dextran gradients for preparation of highly purified plasma membranes and mitochondria in high, equivalent yields from normal and SV-40 transformed lymphocytes was developed. Using the affinity-density perturbation principle, a practical technique was devised using albumin coated polyacrylate beads derivatized with specific ligands. Quantitative and quantative differences between plasma membranes from normal and GD248 cells were found. Non-glycosylated plasma membrane proteins of GD248 cells are more highly amidated than membrane proteins of normal cells. In contrast the glycoproteins are more highly sialytated. At least three protein antigens in GD248 membranes were found which were lacking in membranes of normal cells. It further appears that these antigens are also present in GD248 mitochondria. Using scanning EM lymphoid cells were shown to change surface topology upon contact with inert surfaces, accompanied by alteration in cAMP metabolism and amino acid transport. Techniques have been developed for analysis of purified membranes. Also techniques have been developed for measuring lipid-protein interactions in lymphoid cell plasma membranes.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $88,147

CONTRACT RESEARCH SUMMARY

Title: Mechanisms of Lymphoid Cell Differentiation

Principal Investigator: Ross S. Basch, M.D.
Name/Address New York University Medical Center
Performing New York, New York
Organization:

Contract Number: NO1-CB-43927
Starting Date: 5/1/74 Expiration Date: 4/30/76

Goal: To increase understanding of lymphoid cell differentiation, which is fundamental in investigations of immunologic responses to tumors.

Approach: The contractor will attempt to isolate, identify and determine the state of maturation of thymin-sensitive pre-T cells and to establish the mechanism through which thymin acts to convert these to cells resembling intra-thymic T cells.

Progress: Quantitative assays for the hematopoietic precursor of thymocytes were developed. These assays have been used to study the kinetics of the appearance of progeny of transfused bone marrow, and also studied were spleen cells in the thymus of irradiated mice. The thymocyte precursor developes thymic alloantigens when incubated with thymopoietin and apparently contains terminal deoxyribonucleotidyl transferase. It is relatively X-ray resistant and cortisone resistant. Further characterization of the surface properties of these cells was done and it was determined that the precursor is Ig, Thy-1 and CRL negative. The precursor has at least two antigens on its surface which react with rabbit antisera against mouse brain. One of these is shared with mature thymocytes while the other appears to be related to the antigen present on CFU. Neither the precursor or its immediate progeny can replace mature T cells in an assay for cell cooperativity.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $49,893

CONTRACT RESEARCH SUMMARY

Title: Characterization of Purified Thymic Products Promoting Lymphocyte Differentiation

Principal Investigator: Dr. Gideon Goldstein
Name/Address New York University Medical
Performing Center
Organization: New York, New York

Contract Number: N01-CB-53868
Starting Date: 8/15/74 Expiration Date: 8/14/76

Goal: To characterize thymic factors which affect differentiation of lymphocytes.

Approach: Thymopoetin, the factor originally described by this investigator is being chemically characterized.

Progress: Progress has been made in scaling up the production of Thymopoietin I and II; the investigators have determined the complete amino acid sequence of II and found the first 28 amino acid residues in I are identical to those in II. They have synthesized a biologically active peptide (13 amino acids) corresponding to residues #29-41 in thymopoietin II and have made crystals of thymopoietin II. They have also described the elucidation of the complete amino acid sequence of ubiquitin from bovine thymus (isolated as a side product in thymopoietin purification) and from human thymus and have found it to be identical -- i.e., the same 74 amino acid polypeptide.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date: 6/74
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $101,233

CONTRACT RESEARCH SUMMARY

Title: Liposome Model System

Principal Investigator: Dr. Arnold Sanderson
Name/Address Queen Victoria Hospital
Performing McIndoe Research Unit
Organization: East Grinstead, Sussex, England

Contract Number: NO1-CB-53876
Starting Date: 6/16/75 Expiration Date: 6/15/77

Goal: Elucidation of mechanisms whereby a host succeeds or fails in the elimination of cells bearing foreign antigenic markers (tumor or normal).

Approach: Liposomes will be manufactured with target antigens incorporated into their surface membranes and will contain a substance whose release will be indicative of damage. Investigations will be performed on antibody-complement lysis, lymphocyte dependent antibody lysis and antibody independent cell-mediated lysis.

Progress: New Contract.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $20,000 (for entire 2 year contract)

CONTRACT RESEARCH SUMMARY

Title: Detection of Antigen-Binding Activity of Transplantable T-Cell Tumors

Principal Investigator: Oliver A. Roholt, Ph.D.
Name/Address Health Research Inc.
Performing Roswell Park Memorial Institute
Organization: Buffalo, New York 14203

Contract Number: NO1-CB-43924
Starting Date: 6/16/74 Expiration Date: 6/15/76

Goal: To develop animal models of transplantable T-cell tumors possessing known specific antigen binding capacity.

Approach: The contractor will perform a pilot study, screening a minimum of 15 transplantable T-cell tumors for specific antigen-binding sites. The technique used for detection of antigen binding will be the paired iodination procedure.

Progress: Assays to detect antigen binding by transplantable mouse T-Cell tumors were done on sixteen tumor lines, once or several times, and under different conditions of time and temperature with up to 26 different iodinated antigens. Only a few are suggestive of specific binding but not reproducibly and these are involving the NIP and DNP haptenic groups.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $36,904

CONTRACT RESEARCH SUMMARY

Title: Facility for Supplying Immune Related Cell Lines

Principal Investigator:
Name/Address
Performing
Organization: Dr. Melvin Cohn
The Salk Institute
San Diego, California 92112

Contract Number: N01-CB-23886
Starting Date: 2/26/74 Expiration Date: 2/25/76

Goal: To establish a library of lymphoma and myeloma tumor cell lines and make these available to investigators.

Approach: The contractor will catalog the available cell lines in his laboratory and characterize them as regards their lymphoma and myeloma characteristics. The results will be put into a catalog and either frozen cell lines or mice carrying the appropriate tumors will be made available to investigators on demand.

Progress: The current catalog lists 102 items which are handled routinely and greater than 250 catalogs have been distributed. Since the April printing. The rate of requests for cells and tumor lines has been at the rate of 400 per year.

Significance for Cancer Research (NCP Objective 6 Approach 1) Experimental cancer immunology studies require cell lines and tumors of known characteristics. This contract provides a national resource which supports cancer research throughout the country.

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date: April 1972
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $96,115

CONTRACT RESEARCH SUMMARY

Title: Supramolecular Organization of Normal and Tumor Cell Surfaces

Principal Investigator:
Name/Address
Performing
Organization

Garth L. Nicholson, Ph.D.
The Salk Institute for Biological Studies
P. O. Box 1809
San Diego, California

Contract Number: NO1-CB-33879
Starting Date: 6/25/73
Expiration Date: 9/24/76

Goal: To examine quantity and spatial organization of cell surface antigens and saccharides in normal and malignant cells.

Approach: Normal and transfored cells in tussue culture will be reacted with radioisotope labeled antibodies and lectins and then studies with electron microscopic and cell fractionation procedures.

Progress: 1)Studies show that the lectin receptors in normal cells may be restrained from patching by underlying microfilaments, which are largely absent in transformed cells. 2)Sialic acid residues, which terminate many cell surfaces oligosaccharide, are largely added on to D gal residues in the sugar chains of transformed and normal fibroblasts. 3)A technique for affinity purification of neuraminidase, thereby freeing it of contaminating proteases, was developed. 4)The effect of 12 detergents on the binding activity of 8 lectins to purify detergent solubilized membrane glycoproteins. 5)Studies indicate that polyanionic sites on cell surfaces are linked to lectin receptor sites, since the former undergo topographic redistribution after lectin treatment. 6)Ricin kills transformed fibroblasts by inhibiting protein synthesis subsequent to endocytosis of surface bound lectin.

Significance for Cancer Research (NCP Objective 3 Approach 5)
Understanding cell surface changes underlies many approaches to cancer prevention.

Project Officer: Mrs. Judith Whalen
Program: Immunobiology
Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $146,213

CONTRACT RESEARCH SUMMARY

Title: Investigations of the Nature and Function of Immune-Related Cells in Tumor Masses

Principal Investigator: Stephen W. Russell, DVM, Ph.D.
Name/Address Performing Organization: Scripps Clinic and Research Foundation
La Jolla, California 92037

Contract Number: N01-CB-44001
Starting Date: 6/1/74 Expiration Date: 5/31/76

Goal: To provide information about the numbers of immune-related cells in tumor masses, their identification, function in the tumor mass and factors influencing their movement.

Approach: The contractor will undertake precise identification and quantitation of immune-related cells in neoplasms. An attempt will be made to develop improved methods for use in identification and quantitation of immune-related cells in neoplasms and improved methods of tumor disaggregation.

Progress: The technology to identify and recover the Immune Related Cell (IRC) in regressing neoplasms to assess their functional activities in vitro has been developed. Improved methods of disaggregating tumors were developed, the use of collagenase and DNAse being the choice method. Methods of identifying and quantifying IRC types recovered from neoplasms were developed. Efforts have progressed to enrich the two most common types of IRC thus far found in tumors, macrophages and T lymphocytes. Purification of T lymphocytes from disaggregated tumors on Ficoll isokinetic gradients is proceeding.

Significance for Cancer Research (NCP Objective 4 Approach 1)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $90,000

CONTRACT RESEARCH SUMMARY

Title: Techniques for In Vitro Sensitization of Human Lymphocytes

Principal Investigator: Dr. Leonard Chess
Name/Address Sidney Farber Cancer Center
Performing 35 Binney Street
Organization Boston, Massachusetts 02115

Contract Number: NO1-CB-53881
Starting Date: 5/1/75 Expiration Date: 4/30/76

Goal: Develop or improve techniques for in vitro sensitization of human lymphocytes against syngeneic or autologous tumor associated antigens in order to increase capability for studying cellular interactions involved in the production of cytotoxic cells and ultimately aid in designing new therapeutic approaches to cancer.

Approach: Investigation of sensitization of human lymphocytes to syngeneic and autologous leukemic and solid tumor cells using lymphocytes from both patients and HLA-MLC matched siblings.

Progress: Progress has continued in basic techniques required for in vitro sensitization of human lymphocytes to foreign cell surface antigens including development of a micro-technique for in vitro sensitization using only $2x10^5$ responding cells and an antigen-specific blocking method for cell mediated lympholysis using unlabeled targets during effector phase. Both proliferative and cytotoxic response after in vitro sensitization were studied in experiments using lymphocytes of leukemia patients and from HLA-MLC matched siblings co-cultivated with syngeneic mitomycin-treated leukemic blasts. Experiments were done using mitomycin treated solid tumor cells as stimulating cells for generation of cytotoxic lymphocytes. Most solid tumor cells did not induce generation of killer lymphocytes even in allogeneic normal responding cell populations but there were a few instances of tumor cells from both pleural and peritoneal effusions inducing generation of killer cell activity in allogeneic and autologous lymphocyte populations. Further studies are being done on this phonomenon.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Mrs. Judith Whalen
Program: Immunobiolog Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $56,512

CONTRACT RESEARCH SUMMARY

Title: Human Tumor-Associated Antigens and Corresponding Antibodies

Principal Investigator:
Name/Address
Performing
Organization:

Dr. Yashar Hirshaut
Sloan-Kettering Institute for Cancer Research
410 East 68th Street
New York, New York 10021

Contract Number: N01-CB-53942
Starting Date: 5/30/75 Expiration Date: 6/29/76

Goal: To elucidate that aspect of the immune response to a tumor that leads to production of circulating antibody to cell surface antigens arising after normal cells transform into malignant cells; to aid in development of diagnostic tests and therapeutic approaches to cancer.

Approach: Investigation of possible new tumor-related antigens on sarcomas via membrane immunofluorescence and characterization of these and other markers on various tumor cells.

Progress: New Contract.

Significance for Cancer Research (NCP Objective 3 Approach 4)
Identification and study of tumor-associated antigens is an essential part of the Baseline Information Flow of the Tumor Immunology Program.

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $73,217

CONTRACT RESEARCH SUMMARY

Title: Automated Systems for HLA Typing

Principal Investigator: Dr. F. Carl Grumet
Name/Address Stanford University
Performing Stanford, California 94305
Organization:

Contract Number: NO1-CB-53935
Starting Date: 5/16/75 Expiration Date: 6/15/76

Goal: Refinement and development of assays for identification of antigens.

Approach: Complete development of an automated system for HLA typing utilizing fluorochromesia microcytoxicity and evaluate the system at the Stanford Medical Center.

Progress: New Contract.

Significance for Cancer Research (NCP Objective 5 Approach 3)
To study cell surfaces and cell membranes.

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $36,229

CONTRACT RESEARCH SUMMARY

Title: Recombinant Inbred Strains of Mice for Analysis of Ir-1, Rgv-1 and Linked Genes

Principal Investigator: Hugh O. McDevitt, M.D.
Name/Address Performing Organization: Stanford University School of Medicine
Stanford, California

Contract Number: NO1-CB-43941
Starting Date: 3/1/74 Expiration Date: 2/29/76

Goal: To develop and test mice bearing recombinant H-2 alleles in relation to analysis of the immune response.

Approach: The contractor will develop and maintain large breeding colonies of inbred mice for use in production and testing of antisera directed against a variety of know H-2 recombinants.

Progress: Over the past year the mouse production facility has been increased as well as the breeding and production stocks so that extensive experiments have been undertaken to produce anti-Ia antisera in a variety of combinations. Due to availability of the antisera many studies on Ir gene function were performed.

Significance for Cancer Research (NCP Objective 2 Approach 3)
Studies of genetically influenced immune responses are an important part of the baseline information flow of the Tumor Immunology Contract Program.

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date: 2/74
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $79,977

CONTRACT RESEARCH SUMMARY

Title: Nature of Interactions Between Tumor Cells and Immunoglobulins

Principal Investigator: Dr. Isaac P. Witz
Name/Address Tel Aviv University
Performing Tel Aviv, Israel
Organization:

Contract Number: NO1-CB-43858
Starting Date: 8/15/73 Expiration Date: 8/14/76

Goal: Role of natural and tumor specific immunoglobulin in coating of tumor cells will be examined in relation to antibody and lymphocyte mediated cytotoxicity.

Approach: The contractor will (1) prepare purified Ig from acid eluates of coated tumor cells; (2) examine ascites T lymphoma, mammary carcinoma, and carcinogen-induced sarcoma cells for complement-dependent cytotoxicity, lymphocyte-mediated cytotoxicity and antibody-mediated cytotoxicity; (3) utilize mice immunized with noncross-reactive antigens and then challenged with ascites tumor cells to examine whether these cells show the capacity to bind the radiolabeled immunizing antigens; and (4) study the surface binding capacity of cultured tumor cells for various immunoglobulin fractions.

Progress: Progress was made in determining optimum buffer systems for elution of antibody or immunoglobulin or tumor cell surfaces and in characterization of acid eluates from solid and ascites murine tumor cells. Tumor eluates were capable of increasing the plating efficiency of tumor cells, some enhanced ^{51}Cr release from tumor cells by lymphocytes. Tumor eluates and purified IgG fractions can block or stimulate incorporation of thymidine into syngeneic lymphogeneic antisera. A correlation between the degree of IgG coating and the sensitivity to complement, between these properties and the antigenic expression of the cells was found. Cells in non-lymphoid and lymphoid mouse tumors were found to bind unrelated antibodies such as those against ovalbumin (OA).

Significance for Cancer Research (NCP Objective 3 Approach 4)
The capacity of the immune system to recognize and eliminate transformed cells may depend on and be modified by immunoglobulins.

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $52,958

CONTRACT RESEARCH SUMMARY

Title: Specificity of Antigen-Binding Receptors on T-Cells

Principal Investigator: Dr. Paul H. Maurer
Name/Address Jefferson Medical College of
Performing Thomas Jefferson University
Organization: Philadelphia, Pennsylvania 19107

Contract Number: NO1-CB-43999
Starting Date: 5/1/74 Expiration Date: 5/15/76

Goal: To investigate the properties of antigen-binding receptors on antigen-activated T lymphocytes.

Approach: The contractor will study binding of T cells and B cells with cross-reacting immunogens from immune responder mice, immunized non-responder mice and appropriate control non-immunized mice.

Progress: Stimulation experiments showed that with the polymer GAT as immunogen purified T cells were stimulated best with the homologous polymer and "cross stimulated" with a related polymer $G^{60}A^{40}$, Using GLO^{5} polymer, however, the T cells responded to a closely related polymer GLT^{5} but not to related polymers GLA^{5} and $G^{60}L^{40}$. The T cells needed normal mouse macrophages added to respond to the antigens. The T cell stimulation specificity appears to be quite different from that of the antibody. The ability of lymphocyte plasma membrane fragments or their solubilized components to bind $^{125}I\text{-}GAT^{10}$ has been investigated extensively.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $54,000

CONTRACT RESEARCH SUMMARY

Title: Mechanisms of Lymphoid Cell Differentiation

Principal Investigator: Paavo Toivanen, M.D.
Name/Address Performing Organization: Turku University Medical School
Turku, Finland

Contract Number: NO1-CB-43971
Starting Date: 6/1/74 Expiration Date: 6/30/76

Goal: To increase understanding of lymphoid cell differentiation, which is fundamental in investigations of immunologic responses to tumors.

Approach: The contractor will carry out studies on origin and differentiation sites of pre-bursal stem cells in chickens and on mechanisms of T cell differentiation during early ontogency in man and sheep.

Progress: The contractor demonstrated that, as a differentiation site of the B cell linkage, the bursa precedes the bone marrow during ontogeny. The role of the yolk sac as the first generator of lymphoid stem cells is questionable on the basis of these experiments. Using induced immunodeficiencies the contractor showed that testosterone destroys, in contrast to cyclophosphamide, the capacity of the bursa to serve as a differentiation site for B cells. The activity of alkaline phosphotase in the developing bursa is inhibited by testosterone but not by cyclophosphamide. By using transplantation of "empty" splenic stromas, they have demonstrated that germinal center formation in the spleen is not dependent on histocompatibility between the stromal and lymphoic cells. In studies on mechanisms of T cells differentiation during early ontogeny in man and sheep, the contractor determined that the capacity for immunologically specific effector cells for cell-mediated immunity is found in man fully developed at full term birth.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: (Approximately $59,000)

CONTRACT RESEARCH SUMMARY

Title: Biochemistry of Normal and Tumor Cell Surface Antigens

Principal Investigator: Dr. J. Claude Bennett
Name/Address Performing Organization: University of Alabama in Birmingham
Birmingham, Alabama 35294

Contract Number: NO1-CB-44009
Starting Date: 6/1/74 Expiration Date: 5/31/76

Goal: To isolate, purify and chemically characterize cell surface antigens.

Approach: Using the TL antigens system of the mouse, the contractor will prepare and purify cell surface antigens and perform relevant biochemical studies. An attempt will be made to develop methodologies with wide applicability in studies of cell surface macromolecules.

Progress: The mass Cell Culture Center was assembled and now 10^{12} cells per week can be produced. Several murine lymphoblastoid cell lines were adapted for growth within this setting. Techniques were developed for papain solubilization of the TL antigen, the definition of its subunit structure, the definition of sites and mechanism of cleavage from the cell surface. Progress was made on a more accurate assay for evaluation of cell surface antigens in the presence of detergent, and on development of sensitive biochemical techniques for the routine sequencing of peptides at the 25 nanomole level and even ability to do sequencing at the 10 nanomole level.

Significance for Cancer Researchf (NCP Objective 3 Approach 5)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $97,682

CONTRACT RESEARCH SUMMARY

Title: Analysis of Serum Requirements for In Vitro Immunological Studies

Principal Investigator: Robert I. Mishell, M.D.
Name/Address Performing Organization: University of California, Berkeley
Berkeley, California 94720

Contract Number: NO1-CB-23883
Starting Date: 6/28/72 Expiration Date: 8/31/75

Goal: To define serum requirements for immunological studies employing tissue culture techniques.

Approach: Using the mouse spleen in vitro immune response as an assay, sera obtained from several species will be examined for their capacity to substitute for fetal bovine sera. The latter are in very limited supply. In addition, attempts will be made to explore the use of bacterial endotoxin and other agents for their capacity to restore batches of sera that are not useful in the in vitro assays. Furthermore, preliminary fractionation and characterization of both toxic and growth promoting substances will be carried out.

Progress: 101 samples of FBS were screened for ability to support primary anti-sheep red blood responses in vitro. In addition, sera were screened for ability to support responses to LPS and Con A. There was no clear correlation between the serum effects on humoral responses, mitogen responses, and "background" proliferation of cells. Gram negative bacteria were isolated from one good lot of FBS and factors from these bacteria converted deficient sera to good sera. It was also found that aseptically collected fetal calf sera do not support in vitro immune responses. Little progress was made in development of in vitro immune protection against tumors, presumably because of in vitro development of suppressor T cells. There were some technical difficulties encountered in testing sera for ability to support cellular immune responses.

Significance for Cancer Research (NCP Objective 4 Approach 1)
Most in vitro studies of cellular immune processes depend on added serum. It is essential that sources of such sera be standardized in order to properly evaluate cancer patients' responses to therapy.

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date: 4/25/72
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $60,000

CONTRACT RESEARCH SUMMARY

Title: Genetic Control of Immune Responses in Relation to Cancer

Principal Investigator: Dr. Eli E. Sercarz
Name/Address University of California
Performing Los Angeles, California 90024
Organization:

Contract Number: NO1-CB-43972
Starting Date: 6/01/74 Expiration Date: 10/31/76

Goal: To identify single genes and/or polygenic systems involved in immune responses and to determine their relationship to tumor immunity.

Approach: The contractor will study the mechanisms by which the immune response to gallinaceous lysozymes is regulated at the genetic level in the mouse.

Progress: At least four levels at which genetic control is exerted in the $H\text{-}2^{b}$ mouse have been found;

1. primary control determining whether or not to respond is found in the Ir-GEL locus (mapping in the I-A or possibly K region of the H-2 complex)

2. modulating genes (mapping between the end of I-B and D within H-2) which allow some responsiveness despite a negative allele at I-A

3. a dominant effect (outside H-2) reducing the quantitative level of response about ten-fold and

4. non H-2 linked loci presumably controlling V region specificities

It appears that the balance between regulatory suppressor cells and cooperating helper cells varies in different lymphoid compartments of the mouse and in some way the immunogeneic decision is made on the basis of a key determinent present or absent on a particular lysozyme.

Significance for Cancer Research (NCP Objective 2 Approach 3)

Project Officer: Ms. Judith Magnotta

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $89,786

CONTRACT RESEARCH SUMMARY

Title: Techniques for In Vitro Sensitization of Human Lymphocytes

Principal Investigator:
Name/Address
Performing
Organization

Paul Terasaki, Ph.D.
University of California
Los Angeles, California 90024

Contract Number: NO1-CB-43933
Starting Date: 6/1/74 Expiration Date: 10/31/75

Goal: To develop improved methods for in vitro sensitization of human lymphocytes, directed at producing high yields of cytotoxic cells.

Approach: The contractor will evaluate the efficacy of various immunization methods, measuring release of ^{51}Cr from cultured human tumor cells. Variables will include target cell types, treatment of immunizing cells, types of responding cells, adjuvants, and culture conditions.

Progress: Attempts have been made to optimize conditions under which maximal in vitro sensitization can be obtained, and a number of variables have been studied. Efforts have been concentrated on using long term tumor cell culture lines as target cells with variable results. Various types of treatments of sensitizing cells were also employed, and mitomycin treatment was found to be effective. Variations in lymphocyte responsiveness to target cells were also studied.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $22,488

CONTRACT RESEARCH SUMMARY

Title: Selective Stimulation or Suppression of Humoral or Cellular Immunologic Responses

Principal Investigator: Donald A. Rowley, M.D.
Name/Address University of Chicago
Performing Chicago, Illinois 60637
Organization:

Contract Number: NO1-CB-43998
Starting Date: 6/1/74 Expiration Date: 5/31/76

Goal: To facilitate studies of the mechanisms involved in immunologic responses to tumor cells by separating components of the response.

Approach: The contractor will prepare anti-receptor antibody (ARA) and measure the effect of ARA on immune responses.

Progress: In mice autogenously produced anti-receptor antibody (ARA) suppresses a primary complementary antibody response but not a secondary or existing complementary antibody response. ARA reversibly blocks lymphocyte receptors from adults but irreversibly blocks receptors or eliminates receptor-bearing cells in neonates. A transplantable plasmacytoma induces a transient specific ARA response. Mice immunized with mitomycin-treated plasmacytoma cells produce ARA but don't develop tumor; these mice can survive longer than untreated mice when inoculated with viable plasmacytoma. In rats ARA directed against receptors for allo-antigens dependent cytotoxicity in vitro and such antibody suppresses GVH reactions. By recloning techniques, two different sublines of a cell line secreting Ig have been produced. One secretes little and one secretes abundant Ig, both of which appear to have equivalent density of membrane antigen-specific receptors. Preliminary experiments show that a preparation of ARA reducing the cloning efficiency of the parental line of cells.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $53,039

CONTRACT RESEARCH SUMMARY

Title: Studies by Which Tumors Avoid Destruction by the Immune System

Principal Investigator: Dr. Eugene M. Lance
Name/Address University of Hawaii
Performing 2444 Dole Street
Organization: Honolulu, Hawaii 96822

Contract Number: NO1-CB-53880
Starting Date: 8/16/75 Expiration Date: 8/15/76

Goal: To determine which mechanisms are important in preventing an immune response from effectively destroying tumor cells.

Approach: The contractor will investigate the elimination of lymphocyte trapping in response to antigens and adjuvant by cell-free ascitic fluid of animals with intraperitoneal neoplasms. The resultant suppression of immune responses will be studied.

Progress: New Contract

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $69,877

CONTRACT RESEARCH SUMMARY

Title: T-Memory Cell Properties Primed to Histoincompatible and Syngeneic Tumor Cells

Principal Investigator: Dr. Pekka J. Hayry
Name/Address University of Helsinki
Performing Helsinki, Finland
Organization:

Contract Number: NO1-CB-63977
Starting Date: 7/16/75 Expiration Date: 7/15/77

Goal: Elucidation of properties of T-memory cells to histoincompatible cells and to syngeneic tumor cells.

Approach: The contractor will study restrictions on specificity in secondary responses to various allogeneic histoincompatible cells. The contractor will also study homing, lifetime, and recirculating capacity of secondary T-lymphocytes plus mechanisms for non-responsiveness of secondary T-lymphocytes to T-mitogens.

Progress: New Contract

Significance for Cancer Research (NCP Objective 4 Approach 1)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $20,000 (for entire 2 year contract)

CONTRACT RESEARCH SUMMARY

Title: Improved Detection and Development of H-2 Recombinant Strains

Principal Investigator: Dr. Donald C. Schreffler
Name/Address University of Michigan
Performing Medical School
Organization: Ann Arbor, Michigan 48104

Contract Number: NO1-CB-43936
Starting Date: 6/1/74 Expiration Date: 8/31/75

Goal: To develop improved methods for studying mutation and recombination within the H-2 locus of the mouse, as a model for studying membrane changes in carcinogen or virus induced cancers.

Approach: The contractor will develop an extended series of H-2 recombinant or mutant congenic strains developed from a few key haplotypes. Screening will be done initially by serologic techniques, skin grafting and MLR, with such techniques compared to establish the most effective methods.

Progress: Comparisons of efficiencies and sensitivities of 3 methods of screening for H-2 recombinants are underway.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $18,865

CONTRACT RESEARCH SUMMARY

Title: Selective Stimulation or Suppression of Humoral or Cellular Immunologic Responses

Principal Investigator: Dr. W. J. Cromarthie
Name/Address University of North Carolina
Performing Chapel Hill, North Carolina
Organization:

Contract Number: NO1-CB-43997
Starting Date: 5/16/74 Expiration Date: 11/15/76

Goal: To facilitate studies of the mechanisms involved in immunologic responses to tumor cells by separating components of the response.

Approach: The contractor will study the effects on the immune response of defined components of group A streptococcal cells and determine whether these can affect tumorigenesis or tumor growth.

Progress: Effects of defined group A streptococcal cell components on the immune system have been studied and it has been found that either stimulation or depression of immune cells in culture can be evoked, depending upon the dosage of the streptococcal membrane preparation used.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $49,969

CONTRACT RESEARCH SUMMARY

Title: Biochemistry of Normal and Tumor Cell Surface Antigens

Principal Investigator: Sen-itoroh Hakomori, M.D., Ph.D.
Name/Address University of Washington
Performing Dept. of Pathobiology
Organization: Seattle, Washington 98105

Contract Number: NO1-CB-43920
Starting Date: 6/1/74 Expiration Date: 5/31/76

Goal: To isolate, purify and chemically characterize cell surface antigens.

Approach: The contractor will isolate and chemically characterize "galactoprotein a" "ceramide X" and sialylgalactosyl or sialylgalactosaminyl protein showing specific label for tumors and prepare, purify and characterize antibodies directed against such surface labelled components.

Progress: The presence of a large externally labelled glycoprotein on non-malignant cell lines and its absence from malignant lines has been studied and studies on somatic hybrids between such lines have been initiated. A major advance was the isolation and characterization of a unique surface-labelled glycolipid present in transformed hamster cells.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $59,150

CONTRACT RESEARCH SUMMARY

Title: Organization and Dynamics of Cell Surface Membrane Components

Principal Investigator: Dr. E. D. Kiehn
Name/Address Performing Organization: University of Washington
Seattle, Washington 98105

Contract Number: N01-CB-43987
Starting Date: 6/1/75 Expiration Date: 5/31/76

Goal: To increase understanding of the organization and dynamics of macromolecular components of normal and malignant cell surfaces.

Approach: The contractor will study cell lines transformed by wild type and mutant viruses in comparison to their normal counterparts, investigating regulation of growth levels in vitro and control of cell surface morphology.

Progress: Transformed cells have been shown to become rounded and show increased surface blebbing. Enzymes active on cell surface carbohydrates did not produce such blebbing on normal cells although proteases did. A number of 3T3-type cell lines derived from embryos of Wistar-Furth, B/N, and Fischer rat strains have been characterized. Both SV40 and polyoma transformants have been isolated from some of these lines and comparisons have been made with parent lines regarding doubling times and saturation density in low and high serum, growth in agar, karyotype and in morphological properties. "Normal" rat lines have been characterized with particular attention to plasminogen activation. Studies have continued in the interrelationships of the various in vitro criteria of transformation utilizing thermosensitive mutants of transformed cells, with particular attention to the nature of anchorage-dependence. Studies continue on morphological changes accompanying transformation. Two levels of alteration have been tentatively defined: "intrinsic" changes (subtle and independent of plasminogen activation) and "extrinsic" changes (dramatic and dependent on activation of serum plasminogen for their expression).

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $27,884

CONTRACT RESEARCH SUMMARY

Title: Biochemistry of Normal and Tumor Cell Surface Antigens

Principal Investigator: Dr. Oliver Smithies
Name/Address University of Wisconsin
Performing Madison, Wisconsin 53706
Organization:

Contract Number: NO1-CB-43986
Starting Date: 6/28/74 Expiration Date: 6/27/76

Goal: To isolate, purify and chemically characterize cell surface antigens.

Approach: The contractor will work on development of improved methods of determining primary amino acid sequences, suitable for use in sequencing normal and tumor cell surface antigens. β_2 microglobulin and other model proteins will be employed.

Progress: The contractor showed that preliminary amino acid sequences can be obtained of a cell surface antigen other than β_2m using only radiochemical procedures. This involved: refining labelling procedures to be suitable for small scale tissue culture labelling of HL-A antigens to a great enough specific activity; expanding solubilization and purification procedures; and doing physical and chemical analyses of the purified product to determine they were HL-A antigens. Also, a partial amino acid sequence was obtained on mouse β_2m and determination was made of the chromosomal linkage of the structural gene for β_2m.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $68,601

CONTRACT RESEARCH SUMMARY

Title: Granulocyte and Macrophage Proliferation Control in Leukemia and Related Neoplasma

Principal Investigator: Donald Metcalf, M.D.
Name/Address Performing Organization: The Walter and Eliza Hall Institute of Medical Research
Victoria 3050, Austrailia

Contract Number: NO1-CB-33854
Starting Date: 6/20/73 Expiration Date: 10/19/76

Goal: To examine the regulator substance known as colony stimulating factor (CSF) in relation to its formation, mechanisms of action, and associations with cancer.

Approach: 1) Improve methods for producing the regulator substance Colony Stimulating Factor (CSF) and study its mode of action on target cells, both normal and neoplastic, 2) study the mechanisms by which antigens stimulate the formation of CSF, 3) evaluate the role of CSF in the regulation of granulopoiesis and monocyte formation in preleukemic and leukemic patients as well as normal humans and animals.

Progress: CSF synthesized in vitro by whole C57BL mouse lung was purified and new techniques were devised to increase yields of CSF_{MLCM} from polyacrylamide gel fractions and to prevent inactivation upon storage of purified CSF_{MLCM}. A system was devised for generating CSF from mouse T-lymphocytes using 2 mercaptoethanol +/- added allogeneic cells. A new serum macromolecular factor was identified which amplifies the activity of CSF in vitro in stimulating granulocyte and macrophage colony formation. This factor was effective in mouse marrow cell cultures but not in human cultures and was detected in serum of all species except fetal calf and perhaps horse. An extensive study was made of the factor present in mouse or human serum which suppresses granulocyte and macrophage colony formation in vitro and has been suggested to be a natural inhibiter for CSF in vivo. Studies were completed on CSF and colony-forming cell levels in mice bearing antigenic tumors. A sequential analysis was made of SJL mice developing myeloid leukemia. A survey analysis was completed on patients with acute and chronic myeloid leukemia. The four basic abnormal growth patterns of acute myeloid leukemia cells in vitro were confirmed as was the correlation between these growth patterns and subsequent response to chemotherapy.

Significance for Cancer Research (NCP Objective 6 Approach 1)
Humoral factors, such as CSF, which regulate cells of the immune system are potentially powerful tools for immunotherapy.

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $127,378

CONTRACT RESEARCH SUMMARY

Title: Transfer Factor and Delayed Type Hypersensitivity in the Mouse

Principal Investigator: Dr. J. F. A. P. Miller
Name/Address Walter and Eliza Hall Institute
Performing Victoria, Australia
Organization:

Contract Number: NO1-CB-63992
Starting Date: 3/01/76 Expiration Date: 2/28/77

Goal: To determine whether a state of specific cellular immunity can be conferred upon naive, unsensitized mice by subcellular material extracted from lymphoid cells of appropriately sensitized mice.

Approach: The contractor will establish a reliable objective method of measuring delayed type hypersensitivity (DTH) reactions in mice and determine if specific cellular immunity can be transferred from sensitized to naive mice by cell extracts (thereby demonstrating transfer factor in mice).

Progress: New Contract

Significance for Cancer Research (NCP Objective 2 Approach 1)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $42,335

CONTRACT RESEARCH SUMMARY

Title: The Role of T and B Lymphocytes in Tumor Immunity

Principal Investigator: Dr. G. J. V. Nossal
Name/Address The Walter and Eliza Hall Institute
Performing of Medical Research
Organization: Victoria 3050, Australia

Contract Number: N01-CB-23889
Starting Date: 10/30/74 Expiration Date: 10/29/76

Goal: The work of this project is to analyze the relative roles of T and B cell mediated immune responses in the rejection of tumors in mice.

Approach: The approach will include an analysis of the mechanisms whereby tumor cells are killed and comparisons of various methods of inducing immune response against tumors. Plasma cell tumor systems will be utilized for at least some of the experiments, for fibrosarcomas, spontaneous reticulum cell sarcomas, paraffin oil induced reticulum cell sarcomas, and other experimental tumors will also be studied. All will be examined from the point of view of establishing optimum methods for producing T cell immunity. A portion of this work will require biophysical separation methods that will be applied to permit separate study of T and B lymphocytes. Techniques presently in hand, as well as new procedures that are under investigation, will be applied to the separation of these cells and then the evaluation of the biologic function of the separated cells in tumor systems. The in vitro activation of T lymphocytes and an analysis of their killer activity will be undertaken. In addition, studies will be made of mechanisms for blockading killer cell activity. The final portion of the contract will deal with the mechanism of action of chemically defined aduvants and their possible role in cancer immunology.

Progress: Tumor growth in syngeneic athymic nude mice and the role of T cell in C Parvum induced tumor regression has been studied. C parvum has been found to considerably inhibit growth of many tumors, and two mechanisms, one involving T cells, appear to be operative. Three procedures for separation of CBA mouse T and B lymphocytes and their subpopulations have been compared. In vitro systems for the induction and assay of cytotoxic lymphocytes reactive against syngeneic tumor specific antigens have been further improved, and the system has been used to study the specificity of anti-tumor responses. In the area of lymphocyte blockade by multivalent antigen or by antigen-antibody complexes progress has been made. Also investigations were done to determine the properties of certain proteins of the plasma membranes of mouse T lymphoma cells, using radioiodination of plasma membrane proteins and direct isolation of the plasma membranes.

Significance for Cancer Research (NCP Objective 6 Approach 4)
Understanding and manipulation of T and B cell populations are crucial to immunotherapy of cancer.

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $135,300

CONTRACT RESEARCH SUMMARY

Title: Detection of Antigen Binding Activity of Transplantable T-Cell Tumors

Principal Investigator: Dr. N. L. Warner
Name/Address Walter and Eliza Hall Institute
Performing Post Office, Royal Melbourne Hospital
Organization: Victoria 3050, Australia

Contract Number: NO1-CB-53878
Starting Date: 6/16/75 Expiration Date: 6/15/76

Goal: Develop animal models of transplantable T-cell tumors possessing known specific antigen binding capacity.

Approach: Screen many T-cell tumors with a range of antigens for possible binding activity, especially using complex protein antigens known to be involved in stimulating normal T cell responses.

Progress: New Contract.

Significance for Cancer Researchf (NCP Objective 3 Approach 1)
Determination of the mechanisms accounting for high degrees of stability of cell functioning.

Project Officer: Mrs. Judith Whalen
Program: Immunobiology
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $61,292

CONTRACT RESEARCH SUMMARY

Title: Techniques for In Vitro Sensitization of Human Lymphocytes

Principal Investigator:
Name/Address
Performing
Organization: Dr. Michael Feldman
Weizmann Institute
Rehovot, Israel

Contract Number: NO1-CB-63979
Starting Date: 9/01/75 Expiration Date: 8/31/76

Goal: To develop methods for in vitro sensitization of lymphocytes against autologous human malignant gastrointestinal tumors.

Approach: The contractor will seek optimal culture conditions for human tumor cells. Sensitization will be tested as a function of procedures for purification of lymphocytes, exposure of cell receptors to antigens, and immunopotentiation by adjuvants.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $69,081

CONTRACT RESEARCH SUMMARY

Title: Studies of the Mechanism by Which Tumors Avoid Destruction by the Immune System

Principal Investigator: Dr. Martin Haas
Name/Address Weizmann Institute
Performing Rehovot, Israel
Organization:

Contract Number: NO1-CB-63982
Starting Date: 9/01/75 Expiration Date: 8/31/77

Goal: Elucidation of mechanisms by which tumors avoid destruction by the immune system.

Approach: The contractor will study the mechanism of tumor enhancement by T lymphocytes. Experiments involving manipulation of tumor-bearing mice to influence the activity of enhancing lymphocytes shall be done.

Progress: New Contract

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $73,000

CONTRACT RESEARCH SUMMARY

Title: Studies of the Mechanisms by Which Tumors Avoid Destruction by the Immune System

Principal Investigator: Dr. Nechama Haran-Ghera
Name/Address Performing Organization: Weizmann Institute, Rehovot, Israel

Contract Number: NO1-CB-43930
Starting Date: 6/1/74 Expiration Date: 5/31/76

Goal: To determine which mechanisms are important in preventing an immune response from effectively destroying tumor cells.

Approach: The contractor will investigate the mechanisms of escape from controlled proliferation to overt leukemia development in C57 BL/6 mice.

Progress: "Preleukemic" cells have been identified among bone marrow cells within several days after leukemogenic treatment although overt disease occurred many months after. Studies on the thymus--preleukemic cell relationship show the presence of the intact thymus was a prerequesite for preleukemic cell formation and/or early proliferation. Apparently qualitative instead of quantitative differences exist between preleukemic-leukemic cells tested at different times during the latent period. Two fundamental phases observed during further proliferation of the preleukemic cells were labeled the "dependent" stage related to specific host environments and the "autonomous" phase. The contractor was unable to isolate preleukemic cells from spleen colonies or by culturing bone marrow cells in vitro Proliferation of preleukemic cells is not reduced following anti-Θ serum; these cells bear RLV antigenic markers, are more resistant to osmotic changes in their milieu than normal thymocytes, and they show poor agglutinability by the peanut lectin in contract to normal thymocytes. Also studies were done to further elucidate the cellular basis for immunity of RLV injected C57 BL/6 mice.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $114,750

CONTRACT RESEARCH SUMMARY

Title: Use of Antibodies for Local Delivery of Drugs in Cancer Chemotherapy

Principal Investigator: Dr. Michael Sela
Name/Address Dr. Ruth Arnon
Performing Weizmann Institute
Organization: Rehovot, Israel

Contract Number: NO1-CB-53958
Starting Date: 6/16/75 Expiration Date: 6/15/76

Goal: Explore possibility of using antibodies reacting selectively with cancer cells as tools to bring drugs covalently attached to them to these cells.

Approach: Testing whether or not drug-antibody conjugates will prevent tumor growth in in vivo systems. Also attempting to develop methods for immunospecific isolation of anti-tumor antibodies.

Progress: New Contract.

Significance for Cancer Research (NCP Objective 6 Approach 1 and 4)
Develop the means to cure cancer patients and control the progress of their cancers.

Project Officer: Mrs. Judith Whalen
Program: Immunobiology
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $29,520

CONTRACT RESEARCH SUMMARY

Title: Characterization of Purified Thymic Products Promoting Lymphocyte Differentiation

Principal Investigator: Dr. Nathan Trainin
Name/Address Weizmann Institute of Science
Performing Rehovot, Israel
Organization:

Contract Number: NOL-CB-53926
Starting Date: 6/16/75 Expiration Date: 6/15/76

Goal: To chemically characterize thymic products or other antigens which have been shown in well-defined in vitro assays to promote differentation of lymphoid cells from precursors.

Approach: Isolation, purification and characterization of thymus hormone as well as elucidation of its antigenic properties. Possible organic synthesis.

Progress: The contractor has demonstrated that THF, a thymic factor they have purified, induces immune maturation of lymphoid cells via an obligatory rise in membranal adenyl cyclase activity, and in intracellular levels of cAMP. The hormonal nature of THF has been established. These biological events are exerted only on thymus and thymus derived cells (T cells). They have developed a procedure for isolation and characterization of the active principle of calf thymus. The active principle of THF is a polypeptide of molecular weight approximately 3000. There is a high proportion of acedic residues such as glutamine and aspartic acids on amino acid analysis.

Significance for Cancer Research (NCP Objective 6 Approach 4
Objective 3 Approach 4)
Investigations of alterations in host immunologic responsiveness is an essential component of the Baseline Information Flow of the Tumor Immunology Program.

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date: 8/21-23,1974
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $65,000

CONTRACT RESEARCH SUMMARY

Title: Effect of Thymic Cells and Factors on Tumors

Principal Investigator: Dr. Nathan Trainin
Name/Address Weizmann Institute of Science
Performing Rehovot, Israel
Organization:

Contract Number: NO1-CB-23890 (Part III)
Starting Date: 6/26/72 Expiration Date: 5/25/76

Goal: To determine whether induction of a hyperthymic state in normal animals affects proliferation of transplanted tumors.

Approach: In thymus studies, a wide range of concentrations of syngeneic thymus cells or crude thymic humoral factors will be tested for ability to alter the proliferation of transplanted tumors.

Progress: Partial inhibition of syngeneic tumor growth in vivo has been achieved by administration of syngeneic lymphocytes sensitized with thymic humoral factor on tumor monolayers.

Significance for Cancer Research (NCP Objective 5, 6 Approach 4, 1)
Specific cell populations will have to be appropriately manipulated to take maximum advantage of the potentialities of immunotherapy.

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date: 8/74
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $234,713

CONTRACT RESEARCH SUMMARY

Title: Selective Stimulation or Suppression of Humoral or Cellular Immunologic Responses

Principal Investigator: Dr. D. C. Edwards
Name/Address Wellcome Research Laboratories
Performing Beckenham, Kent, England
Organization:

Contract Number: NO1-CB-43929
Starting Date: 6/20/74 Expiration Date: 6/19/76

Goal: To facilitate studies of the mechanisms involved in immunologic responses to tumor cells by separating components of the response.

Approach: The contractor will study means for selectively stimulating T cell responses to well-defined antigens. An attempt will be made to distinguish adjuvants which act primarily on T cells and others which act on B cells and to devise methods for linking these adjuvant molecules to antigens and to antibodies.

Progress: The most suitable experimental conditions for linking diphtheria toxin peptide A and Ricin peptide A to antibody IgG through a disulfide bridge have been developed. Ricin peptide A can be prepared on a large scale. This disulfide linked peptide A is available for biological evaluation on a preparative scale. Several specific antisera have been prepared, tested for cytotoxicity and are available for coupling.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $16,160

CONTRACT RESEARCH SUMMARY

Title: Selective Stimulation or Suppression of Humoral or Cellular Immunologic Responses

Principal Investigator: Dr. Richard K. Gershon
Name/Address Performing Organization: Yale University School of Medicine
New Haven, Connecticut 06510

Contract Number: NO1-CB-43994
Starting Date: 6/1/74 Expiration Date: 5/31/76

Goal: To facilitate studies of the mechanisms involved in immunologic responses to tumor cells by separating components of the response.

Approach: The contractor will attempt to identify and characterize subpopulations of lymphocytes with the capacity to suppress responsiveness of other lymphoid cells.

Progress: Progress has been made in studies on factors governing generation of suppressor T cells as follows: An in vitro system for generation of specific suppressor T cells was developed. Antigen dose is a key factor in forming generation of suppressor cells. Low doses favor development of specific helper cells. Following generation of suppressor T cells, further cell interactions are important for activation of these suppressor cells, and the activity of a given population of cells can be caused to change from specific suppression to specific help. Cells become operationally less susceptible to effects of suppressor cells with time after immunization. It is probable that at least part of the suppressor is effected by the events occurring at the macrophage membrane and the contractor has been able under some conditions to block suppression by adding heat killed macrophages or semi-purified macrophage membranes to the cultures.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $71,028

CONTRACT RESEARCH SUMMARY

Title: Detection of Antigen Binding Activity of Transplantable T-Cell Tumors

Principal Investigator: Dr. Frank F. Richards
Name/Address Yale University School of Medicine
Performing 333 Cedar Street
Organization: New Haven, Connecticut 06510

Contract Number: NO1-CB-63993
Starting Date: 9/01/75 Expiration Date: 8/31/76

Goal: To obtain transplantable tumor cell lines of T-cell origin which bind ligands and use these cell lines to understand the structural correlates and biological consequences of ligand binding to T-cells.

Approach: The contractor will screen transplantable tumor lines of T-cell origin for their ability to bind ligands.

Progress: New Contract

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $52,948

CONTRACT RESEARCH SUMMARY

Title: Characterization of Purified Thymic Products Promoting Lymphocyte Differentiation

Principal Investigator: Dr. Byron H. Waksman
Name/Address Yale University School of Medicine
Performing New Haven, Connecticut 06510
Organization:

Contract Number: NO1-CB-43926
Starting Date: 6/16/74 Expiration Date: 11/15/76

Goal: To chemically characterize an agent shown in well defined in vitro assays to promote differentiation of lymphoid cells from precursors.

Approach: The contractor will obtain and purify "lymphocyte activating factor" (LAF) from normal human lymphocytes and test its mitogenic action on mouse thymocytes.

Progress: The contractor has focused on improving the production of "lymphocyte activating factor" (LAF), divising new systems for purification, and evaluating the response of target cells to optimize the assay. A high volume assay system was developed. A macrophage line (P388D1 tumor cells) was established and there is preliminary evidence that this line is able to produce large amounts of LAF. The response of mouse thymocytes to LAF in the assay has been studied as a function of age and also in response to a tumor challenge in older animals. Mitogenic activity of highly purified Rhesus monkey LAF has been demonstrated on thymocytes of that monkey. Studies on 10 patients receiving BCG therapy have shown no change in the ability of their peripheral monocytes to produce LAF before and after treatment. Studies on the role of macrophages in preventing contact inhibition by lymphocytes in cultures with agglutinating mitogens have been done. Possible roles in helper and suppressor events have been observed in cultures of sensitized lymphocyte subpopulations with antigens and mitogens and the study is continuing.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Mrs. Judith Whalen
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $65,118

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Human Peripheral Blood Mononuclear Cells

Principal Investigator: Dr. Leonard Chess
Name/Address Children's Cancer Research Foundation
Performing 35 Binney Street
Organization: Boston, Massachusetts 02115

Contract Number: NO1-CB-43964
Starting Date: 5/16/74 Expiration Date: 5/15/76

Goal: Development of procedures for more effective and reproducible fractionation of mononuclear cells from human peripheral blood.

Approach: Peripheral blood from normal individuals and from patients with carcinomas will be studied. The following cell types will be isolated in suspension with high yields, purity, and viability: T lymphocytes, B lymphocytes, and monocytes. The isolated cells will be characterized for viability, morphology, retention of membrane markers, and retention of immunological functions.

Progress: Various cell separation techniques were extended and developed. These include use of Sephadex G-200 anti-Fab column immunoabsorbent chromatography, rosette sedimentation techniques, the fluorescence activated cell sorter, cell electrophoresis and specific antisera raised to subsets of T cells and null cells. Progress was made regarding functional characterization of isolated lymphocyte subsets in the following areas: proliferative responses, production of the mediators MIF and LIF by isolated human T cells and B cells triggered by specific antigen, cytotoxic responses, and human B cell function with respect to specific antibody production and immunoglobulin synthesis.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $72,226

CONTRACT RESEARCH SUMMARY

Title: Improvement of Methods for Isolation and Preservation of Lymphocytes

Principal Investigator: Dr. Roy Weiner
Name/Address Children's Cancer Research Foundation
Performing 35 Binney Street
Organization: Boston, Massachusetts 02115

Contract Number: NO1-CB-43885
Starting Date: 5/16/74 Expiration Date: 5/15/76

Goal: To develop improved techniques for the preservation of the immunological reactivity of human peripheral blood lymphocytes.

Approach: Lymphocytes will be separated from the peripheral blood of normal individuals and of patients with carcinomas and will be preserved in the frozen state and then tested for retention of their biological properties, to include reproducible immunolobical reactivity.

Progress: Lymphocytes cryopreserved in the first contract year were tested in this second year for effect of dilution temperature and rate of dilution on recovery of structure and function. Warmer dilution temperature resulted in higher numbers of E-rosettes, higher background incorporation of ^{3}H thymidine in MLC and better recovery of CFU-C activity. Slower rate of dilution had no effect on CFU-C activity but tended to increase 3H-thymidine incorporation in MLC. Vial to vial reproducibility of MLC function of cryopreserved cells was tested and was reproducible within 2 S.E.M. of the triplicates of each vial with multiple vials of the same population. Collection of large numbers of lymphocytes was studied using the Haemonetics apparatus. The problem of clumping of thawed cells has been eliminated by depleting the population of platelets. A new technique of electrophoretic light scattering as a measure of electrophoretic mobility was applied to fresh and cryopreserved lymphocytes. Mobility of fresh and frozen lymphocytes was identical indicating no change in membrane charge density.

Significance for Cancer Research (NCP Objective 5 Approach 2)

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $63,384

CONTRACT RESEARCH SUMMARY

Title: Purified Carcinoembryonic Antigen and Antisera

Principal Investigator: Dr. Charles W. Todd
Name/Address City of Hope National Medical Center
Performing Duarte, California 91010
Organization:

Contract Number: NO1-CB-23877
Starting Date: 6/1/72 Expiration Date: 1/31/76

Goal: To provide purified carcinoembryonic antigen and antisera to carcinoembryonic antigen for use in studies involving the immunodiagnosis of cancer.

Approach: The contract will use presently available techniques to purify carcinoembryonic antigen. In addition, the contractor will investigate additional approaches for the preparation and isolation of these materials. Purified antigen will be used to immunize animals and produce antisera of appropriate specificity and strength for use in radioimmunoassay for diagnostic purposes. Purified CEA will be partially characterized from the chemical point of view, and where indicated, fragments will be prepared and their structure determined.

Progress: The contractor provided 32 other investigators with carcinoembryonic antigen (CEA) and/or antisera and conducted workshops on CEA radioimmunoassays involving 34 trainees. The laboratory has been designated by WHO as a collaborating center for CEA standardization. Advances were made in characterization of the CEA molecule. It was demonstrated that most of the sugars (including 100% of fucose and sialic acid could be removed without decreasing inhibitory activity of the molecule in the radioimmunoassay. Peptide fragmentation studies provided evidence that there is only one peptide chain and that there is more than one antigenic determinant on the CEA molecule. Progress was also made toward solving some of the technical problems involved in obtaining the amino acid sequence of the CEA protein.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Knowledge of the structure of CEA might make possible improved assays for detection of this carcinofetal antigen.

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date: 2,18,19/74
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $108,519

CONTRACT RESEARCH SUMMARY

Title: Purification of Human Tumor Associated Antigens

Principal Investigator: Dr. George L. Wright
Name/Address Eastern Virginia Medical Authority
Performing 333 West Freemason Street
Organization: Norfolk, Virginia 23507

Contract Number: NO1-CB-53955
Starting Date: 6/30/75 Expiration Date: 6/29/76

Goal: To isolate, purify and identify human tumor associated antigens.

Approach: Isolate and purify tumor associated antigens from crude tumor extracts and test them for functional activity in assays of humoral and cell-mediated immunity.

Progress: The contractor has been able to prepare 3M KCl extracts of nine tumor specimens. The leukocyte migration inhibition assay has been developed and standardized using the capillary tube method. The T-24 (transitional-cell carcinoma) cell line has been grown in large volumes for antigen processing.

Significance for Cancer Research (NCP Objective 5 Approach 4)
To purify human tumor associated antigens and make these available for diagnostic testing and evaluation in assays for cell-mediated immunity.

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $77,753

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Assays for Circulating Tumor Associated Antigens: CEA

Principal Investigator: Dr. Theodore Hersh
Name/Address Emory University
Performing 1364 Clifton Road, N.E.
Organization: Atlanta, Georgia 30322

Contract Number: NO1-CB-43887
Starting Date: 3/15/74 Expiration Date: 3/14/76

Goal: To determine whether the CEA assay is a useful adjunct in the diagnosis of gastrointestinal cancer.

Approach: Patients who have signs and symptoms suggestive of gastrointestinal cancer will have complete clinical and laboratory evaluation, and in addition CEA values will be determined. Correlation of elevated CEA levels with initial diagnosis of gastrointestinal cancer or development of cancer on subsequent followup will be evaluated.

Progress: To date 1100 patients with one of twelve G.I. signs or symptoms, as described in the contract, have been enrolled in this study. Clinical evaluations for diagnoses were performed and blood was sent coded to NCI for CEA determination. Computer forms have been prepared to enter data for subsequent statistical analysis.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date: 2/14/74, 12/2/75
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $52,322

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Assays of Circulating Tumor Associated Antigens: AFP and HCG

Principal Investigator: Dr. Melvin Moore
Name/Address Emory University
Performing 1364 Clifton Road, N.E.
Organization: Atlanta, Georgia 30322

Contract Number: NO1-CB-43888
Starting Date: 3/15/74 Expiration Date: 3/14/76

Goal: To determine the clinical usefulness of alpha fetoprotein test and test for human chorionic gonadotrophin in the differential diagnosis of testicular cancer.

Approach: Patients with signs or symptoms consistent with the diagnosis of intestinal cancer will be evaluated clinically and will also have AFP and HCG tests performed. Correlation of the test results and diagnosis of cancer will be examined.

Progress: To date 50 patients have been entered into the study. Pathological data and slides have been obtained on all patients for submission to NCI review. Serum marker specimens have been submitted for hCG and AFP analysis. In the preorchiectomy group, five of the eight patients with malignancies had germ cell tumors and all of these five had abnormal marker studies pre-operatively. Three of these five are patients free of disease and now have normal AFP and hCG levels. Two of the patients with non-germ cell tumors had normal hCG and AFP levels prior to orchiectomy. Of 26 patients with testicular malignancy entered on study post-orchiectomy, 10 had active disease and 16 had no clinical evidence of disease. Six of ten patients with active germ cell cancer had positive markers; there were three false negatives. Fifteen of 16 patients with no evidence of disease had normal hCG and AFP values. Markers are not available on one patient in each group.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date: 2/15/74-12/2/76
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $26,545

CONTRACT RESEARCH SUMMARY

Title: Cutaneous Hypersensitivity Antigen Reactions

Principal Investigator: Dr. Ariel Hollinshead
Name/Address Performing Organization: George Washington University
2300 I Street, N.W.
Washington, DC 20037

Contract Number: N01-CB-92176
Starting Date: 6/27/69 Expiration Date: 4/30/76

Goal: To determine whether skin testing for delayed hypersensitivity reactions can be used to measure cellular immunity to tumor-associated antigens. To use the skin test technique as an assay for separation and purification of tumor antigens.

Approach: Extraction and purification of skin reactive antigens from carcinoma of the breast, malignant melanoma, and acute leukemia. Perform skin tests on patients at various stages of disease, to determine relationship of reactivity to clinical course. Provide antigens for possible use in in vitro assays of cell-mediated immunity.

Progress: During the past year the main focus of this project has been the largest scale testing of patients at NIH under a coded protocol with soluble separated fractions prepared from melanoma and breast cancer specimens. The specificity of the delayed hypersensitivity reactions obtained was analyzed in detail, by including tests on patients with the same type of disease and also with other types of cancer. The antigens on primary tumors, metastatic lesions and established tissue culture lines derived from tumors were compared. In addition to tests for delayed hypersensitivity skin reactions, some of the fractions were tested in vitro by the leukocyte migration inhibition assay. These experiments provided preliminary indications of specific reactivity to nanogram concentrations of breast cancer associated antigens. Some of the separated antigens which reacted in cell-mediated immunity assays, were used to immunize rabbits, in an attempt to produce antibodies against the tumor associated antigens.

Significance for Cancer Research (NCP Objective 5 Approach 4) A fundamental problem in tumor immunology is the demonstration of tumor specific antigens in human tumors. This contract provides an opportunity to study delayed skin reactions to antigens.

Project Officer: Dr. Ronald Herberman
Program: Immunology: Immunodiagnosis Site Visit Date: 1/03/74
Technical Review Group: Ad Hoc Intramural Review Committee
Relevance Review Group:
FY76 Funds: $176,383 This contract was recompeted as an immunology support contract and an award is anticipated prior to the expiration of this contract.

CONTRACT RESEARCH SUMMARY

Title: New In Vitro Techniques to Evaluate Cell Mediated Immunity to Intact Tumor Cells

Principal Investigator: Dr. Christopher S. Henney
Name/Address Johns Hopkins University
Performing 725 N. Wolfe Street
Organization: Baltimore, Maryland 21205

Contract Number: NO1-CB-43965
Starting Date: 5/16/74 Expiration Date: 5/15/76

Goal: Development of new in vitro techniques or substantial modifications of existing techniques for the evaluation of cell-mediated immunity to whole tumor cells.

Approach: In syngeneic animal tumor model systems, the use of ^{86}Rb will be compared with the release of $^{51}Chromium$ for measuring cytotoxicity of tumor cells by immune lymphocytes. The use of gluteraldehyde-fixed tumor cells for lymphocyte stimulation will be examined. The conjugation of soluble tumor antigens to sensitive indicator cells for measurement of cell-mediated immunity to tumor antigens will be examined.

Progress: The prime concern of this contract has been to study ways in which in vitro cytolytic assays can be more widely applied to measurement of cell-mediated immune responses to tumor associated antigens. The contractor was unable to establish a reproducible lytic assay system in a syngeneic tumor model. This was shown apparently not to be due to an intrinsic resistance of the fibrosarcoma cells to lytic attack. The mode of preparation of single cell suspensions governs the use of cells from solid tumors as target cells for lytic attack. Also studied were ways to augment the lytic activity of effector T cells prior to in vitro assay.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $76,885

CONTRACT RESEARCH SUMMARY

Title: Study of Detection of Carcinoembryonic Antigen in Humans

Principal Investigator: Dr. Calvin A. Saravis
Name/Address Mallory Institute of Pathology
Performing Boston, Massachusetts 02118
Organization:

Contract Number: NO1-CB-33264
Starting Date: 6/1/73 Expiration Date: 5/31/76

Goal: Investigate colonic tumor-associated antigens.

Approach: Isolate and characterize colonic tumor associated antigens, produce antisera to the antigens, by using tumor tissue fractions and tumor cell membranes, detect tumor-associated enzyme activity.

Progress: Improved methods were developed for zinc glycinate fractionation of tumor cells, membranes, and extracts, thus revealing new tumor marker antigenic sites. Also improved means of producing operationally monospecific anti-tumor marker antisera and for isolation and partial characterization of a new tumor marker were developed. Therefore, large amounts of anti-zinc glycinate marker (ZGM) antisera have been produced as well as large quantities of relatively pure ZGM. ZGM has been found in saline or zinc glycinate extracts of 50% of primary colonic tumors tested as well as in 1/3 of metastatic colonic tumors but was not found in normal human plasma, normal colon, normal liver, normal spleen, various blood group antigens or in grossly non-malignant colonic mucosa adjacent to ZGM containing primary colon tumors. ZGM was distinct from alpha-fetoprotein, alpha-macroglobulin, ferritin-like macromolecules, CEA and from antigens cross-reacting with CEA.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $57,512

CONTRACT RESEARCH SUMMARY

Title: Human Tumor Associated Antigens of the Tract Other than CEA and Alphafetoprotein

Principal Investigator: Dr. Elliot Alpert
Name/Address Massachusetts General Hospital
Performing Boston, Massachusetts 02114
Organization:

Contract Number: NO1-CB-43889
Starting Date: 6/01/74 Expiration Date: 5/31/76

Goal: To search for new tumor associated antigens of the gastrointestinal tract, which may be more useful for early diagnosis than CEA or AFP.

Approach: Identify by immunological methods antigens which may be useful in the detection of carcinoma of the gastrointestinal tract. Employ these methods in screening both biopsy specimens and cell cultures of gastrointestinal tract tumors for new antigens. Characterize new antigens and demonstrate usefulness for large scale testing of patient populations.

Progress: This is a joint collaborative project between Alpert and Schur, although the tasks and responsibilities are explicitly divided between the two investigators according to their areas of expertise and facilities and they have separate budgets. The progress can be summarized on the joint effort:

To date 54 cancer tissues have been extracted or dissociated in "particulate" preparations; 63 normal tissues were collected as controls. Thirty-four pools of antisera have been absorbed with pools of normal plasma serum and tissue or cell extracts. Eluants from immunoabsorbant columns made from two absorbed anti-cancer membrane extract antisera that bind material from tumor extracts, appear as two distinct bands on polyacrylamide gel electrophoresis. Of 34 completely absorbed and tested antisera pools, 5 still contain precipitates to extracts of adenocarcinomas of the pancreas and colon.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: R. Raymond Gantt, M.D.
Program: Immunodiagnosis Site Visit Date: 4/5/74
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $61,436

CONTRACT RESEARCH SUMMARY

Title: Blood Sample Collection Bank as Reservoir for Evaluation of Cancer Detection Tests

Principal Investigator:
Name/Address
Performing
Organization:

Dr. Allan Schutt
Mayo Foundation
200 First Street, S.W.
Rochester, Minnesota 55901

Contract Number: NO1-CB-23879
Starting Date: 6/15/72 Expiration Date: 11/14/76

Goal: To establish and maintain a bank of sera from patients with cancer, with benign diseases and from normal individuals.

Approach: Make necessary serum samples available for evaluation of immunodiagnostic tests for cancer. Serve as a central facility for storage of serum and plasma specimens collected by other contractors in the tumor immunology-immunodiagnosis program.

Progress: Collection of blood samples from Mayo patients with tumors and benign diseases, and from disease-free individuals has been continued. Information on diagnosis, pathologic grade and clinical stage, treatment status, age, sex, time of blood drawing, and other parameters is kept on each sample with appropriate cross-indexing. Mechanisms have been developed and implemented for orderly shipment of screening serum samples from outside project sites. About 3000 vials arrive every two weeks from Minneapolis and Philadelphia sites. A large number of serum panels for evaluating potential immunodiagnostic tests for cancer have been compiled and sent to designated investigators through arrangements with the Immunodiagnosis Program.

Significance for Cancer Research (NCP Objective 5 Approach 4)
This serum bank will provide sera, in sufficiently large quantities and with adequate controls, to efficiently and rapidly evaluate potential immunodiagnostic tests for cancer.

Project Officer: Mrs. Judith Whalen
Program: Immunology: Immunodiagnosis Site Visit Date: 2/19/74
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $105,000

CONTRACT RESEARCH SUMMARY

Title: Techniques Detection Immunofluorescent of Antibodies Against Autologous Human Tumor Cells

Principal Investigator: Dr. Fiorenzo Paronetto
Name/Address Mt. Sinai School of Medicine
Performing 5th Avenue at 100th Street
Organization: New York, New York 10029

Contract Number: NO1-CB-43953
Starting Date: 5/16/74 Expiration Date: 5/15/76

Goal: To identify specific antibodies in the sera of patients with carcinoma of the lung, breast, and colon.

Approach: Sera from cancer patients and from controls will be tested against autologous tumor cells by immunofluorescence assays with viable and fixed tumor cells. Sera for testing will be obtained before and at appropriate intervals after therapy. Specificity of observed reactions will be determined.

Progress: Fluorescent antibody techniques have been used to detect autoantibodies against human colon, lung and breast cancer. Three different procedures have been used (cryostat sections, cell suspensions and cell cultures). Results suggest the presence of antigen-antibody complexes in the tumor. Optimal procedures have been defined for obtaining vital cell suspensions from solid tumors without enzymatic treatment of the tumors. Long-term established cell lines derived from carcinomas of the colon, lung and breast have been grown and optimal conditions under which the cell lines may be used to screen sera of cancer patients for tumor specific autoantibodies have been studied.

Significance for Cancer Research (NCP Objective 5 Approach 2)

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date: 2/3/76
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $84,283

CONTRACT RESEARCH SUMMARY

Title: Purification of Human Tumor Associated Antigens

Principal Investigator: Dr. Arthur Malley
Name/Address Performing Organization: Oregon Regional Primate Research Center
505 N.W. 185th Avenue
Beaverton, Oregon 97005

Contract Number: NO1-CB-53954
Starting Date: 6/16/75 Expiration Date: 6/15/76

Goal: To isolate, purify and identify human tumor associated antigens.

Approach: Isolation of homogeneous preparation of tumor associated antigens from melanoma and squamous cell carcinoma tissue in sufficient quantity to permit biological and chemical characterizations. Also, to test these antigens at various stages of purification for functional activity in assays of humoral and cell-mediated immunity.

Progress: Methods of assaying, extracting and fractionating tumor specific antigens (TSA) have been compared. The leukocyte adherence inhibition test have been modified so as to be reproducible, quantitative and applicable to large scale testing of fractionation products. The LAI was found to correlate well to dermal testing of TSA. Seven extraction procedures were compared on melanoma and squamous cell carcinoma tissues. The 3.5M KCI extraction for 48 hours produced the greates yield of tumor specific antigens.

Significance for Cancer Research (NCP Objective 5 Approach 4)
To purify human tumor associated antigens and make these available for diagnostic testing and evaluation in assays for cell-mediated immunity.

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $66,314

CONTRACT RESEARCH SUMMARY

Title: Collection of Sera from Populations with High Cancer Risk

Principal Investigator: Henry Altschuler, Ph.D.
Name/Address Philadelphia Geriatric Center
Performing 5301 Old York Road
Organization: Philadelphia, Pennsylvania 19141

Contract Number: NO1-CB-33914
Starting Date: 6/25/73 Expiration Date: 10/24/76

Goal: To collect serial serum specimens from populations at high risk of developing malignant disease for the purpose of developing and evaluating immunodiagnostic screening tests for human cancer.

Approach: Stable populations at high risk of developing the more common human cancers, such as colon, lung, breast, prostate, or bladder, will be followed on an annual basis. This will include clinical evaluation and serum collection. The sera will be banked and records will be kept on the patients so that stored sera can be correlated with development of any particular cancer.

Progress: Sera are continually collected from a demographically stable population of high cancer risk age residents of Philadelphia Geriatric Center and as well as others. Complete records are kept on each patient including medical information and follow-up data. Sera are sent to Mayo Clinic for storage under Dr. Schutt's project.

Significance for Cancer Research (NCP Objective 5 Approach 1)
Early detection of cancer by means of appropriate diagnostic testing would permit treatment of cancer at the most favorable time.

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $51,405

CONTRACT RESEARCH SUMMARY

Title: Human Tumor Associated Antigens of the GI Tract Other Than CEA and Alphafetoprotein

Principal Investigator: Dr. Peter H. Schur
Name/Address Robert B. Brigham Hospital
Performing Boston, Massachusetts 02120
Organization:

Contract Number: NO1-CB-43958
Starting Date: 6/1/74 Expiration Date: 5/31/76

Goal: To search for new tumor associated antigens of the gastrointestinal tract, which may be more useful for early diagnosis than CEA or AFP.

Approach: Identify by immunological methods antigens which may be useful in the detection of carcinoma of the gastrointestinal tract. Employ these methods in screening both biopsy specimens and cell cultures of gastrointestinal tract tumors for new antigens. Characterize new antigens and demonstrate usefulness for large scale testing of patient populations.

Progress: This is a joint collaborative project between Alpert and Schur, although the tasks and responsibilities are explicitly divided between the two investigators according to their areas of expertise and facilities and they have separate budgets. The progress can be summarized on the joint effort:

To date 54 cancer tissues have been extracted or dissociated into "particulate" preparations; 63 normal tissues were collected as controls. Thirty-four pools of antisera have been absorbed with pools of normal plasma serum and tissue or cell extracts. Eluants from immunoabsorbant columns made from two, absorbed, anti-cancer membrane extract antisera that bind material from tumor extracts, appear as two distinct bands on polyacrylamide gel electrophoresis. Of 34 completely absorbed and tested antisera pools, 5 still contain precipitates to extracts of adenocarcinomas of the pancreas and colon.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: R. Raymond Gantt, M.D.
Program: Immunodiagnosis Site Visit Date: 4/5/74
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $54,296

CONTRACT RESEARCH SUMMARY

Title: Carcinoembryonic Antigen and Tumor-Specific Antigens in Diagnosis of Cancer

Principal Investigator: Dr. Tsann Ming Chu
Name/Address Roswell Park Memorial Institute
Performing 666 Elm Street
Organization: Buffalo, New York 14203

Contract Number: NO1-CB-33858
Starting Date: 6/25/73 Expiration Date: 10/24/76

Goal: To evaluate the usefulness of carcinoembryonic antigen (CEA) assay in several varieties of human cancer. It will be evaluated for its capacity to 1) monitor recurrence of disease and 2) to measure the effectiveness of therapy.

Approach: This will be done by 1) determining a baseline value on all new patients who may fit into one of these categories, 2) evaluating CEA levels at 4 and 14 days after surgery (with some selected patients having more assays), 3) additional testing will be done at 30 and 60 days and every three months thereafter. For patients receiving radiation therapy, CEA testing will be done at two-week intervals during the therapy and at monthly intervals thereafter. Patients on chemotherapy will be tested at weekly intervals for ten weeks and then months.

Progress: The prognostic and post-operative monitoring capabilities of the CEA assay were compared to pathologic stagings of the patient. Pre-operative CEA determinations were a good prognostic tool comparable to pathologic staging of the surgical specimen. Post-operative CEA monitoring provided the earliest sign of recurrence in 14 of 23 patients and was positive at the time of recurrence determined by other methods in 20 cases (85%) being negative in only 3 cases. A three-year follow-up study of 228 patients with lung cancer was also evaluated. In a significant number of patients increasing levels of CEA may anticipate clinical evidence of progression of disease by several months.

Significance for Cancer Research (NCP Objective 5 Approach 4)
CEA is one of the more promising assays for evaluating effects of therapy on certain cancers.

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $61,645

CONTRACT RESEARCH SUMMARY

Title: Development of Macrophage Electrophoretic Mobility Assay for Malignant Disease

Principal Investigator: Dr. Leonard Weiss
Name/Address Roswell Park Memorial Institute
Performing Health Research, Inc.
Organization: 666 Elm Street
Buffalo, New York 14203

Contract Number: NO1-CB-53901
Starting Date: 6/1/75 Expiration DAte: 5/31/76

Goal: To further evaluate the macrophage electrophoretic mobility (MEM) test, to determine its possible clinical applications, and to either standardize the current assay or develop an improved method for detection of reactivity.

Approach: Attempt to reproduce the MEM test for the diagnosis of malignancy and compare results with macrophage migration inhibition and detachment tests already being conducted.

Progress: A methodology for the macrophage electro-phoretic mobility (MEM) assay for malignant disease was established. Reliable assays showed 66% of the cancer patients gave positive results but no person without cancer gave a positive. Comparison studies are being done between conventional macrophage migration inhibition tests and a novel macrophage detachment assay, using the same materials for assay. Peripheral blood lymphocytes were isolated from cancer patients (mostly GI cancer) and data has been collected from other tests performed on these patients by the Department of Surgery. Analogous experiments have been initiated using animal tumor systems.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $34,212

CONTRACT RESEARCH SUMMARY

Title: Evaluation of an Isomeric Species of Carcinoembryonic Antigen (CEA-S)

Principal Investigator: Dr. Robert M. Nakamura
Name/Address Scripps Clinic and Research Foundation
Performing 476 Prospect Street
Organization: La Jolla, California 92037

Contract Number: NO1-CB-53934
Starting Date: 5/16/75 Expiration Date: 5/15/76

Goal: To evaluate CEA-S in the diagnosis of human cancer.

Approach: Isolate and characterize CEA-S from colon carcinoma; produce anti-sera to CEA-S, develop an assay for CEA-S and evaluate this assay.

Progress: CEA-S can now be consistently isolated from gastrointestinal tumors. The diagnostic efficacy of CEA and CEA-S ligands in radioimmunoassays can be compared without consideration of individual antigenic variation between tumors since two preparations of conventional CEA have been made to compare with CEA-S isolated from the same tumor. An alternate isolation procedure avoiding perchloric acid extraction has yielded CEA that exhibits antigenic activity and purity greater than conventional CEA but less than CEA-S isolated from the same tumor. Antisera to three different preparations of CEA-S and CEA have been produced. A systematic protocol has been developed for evaluation of antisera to different preparations of CEA-S.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $99,945

CONTRACT RESEARCH SUMMARY

Title: Purification of Human Tumor Associated Antigens

Principal Investigator: Dr. Ralph A. Reisfeld
Name/Address Scripps Clinic and Research Foundation
Performing 476 Prospect Street
Organization: La Jolla, California 92037

Contract Number: NO1-CB-53899
Starting Date: 6/1/75 Expiration Date: 5/31/76

Goal: To isolate, purify and identify human tumor associated antigens.

Approach: Purify human tumor associated antigens and make them available for cell-mediated immunity assays and for diagnostic testing.

Progress: Using delayed cutaneous hypersensitivity responses (DCHR), human melanoma-associated antigens solubilized from fresh surgical specimens were shown to be antigenic. Between 60 and 70% of melanoma patients and only 25% of patients with other neoplasms reacted while only 10-15% of melanoma patients reacted to an extract of autologous muscle. Evaluation of lung tumor antigens has been confined so far to 3M KCI extracts of fresh lung cancer and adjacent normal tissues. Only one preparation exhibited some specificity in DCHR tests with lung cancer patients. A twenty-fold purification of the antigenic moiety was achieved and also demonstrated an apparent increase in specificity.

Significance for Cancer Research (NCP Objective 5 Approach 4)
To purify human tumor associated antigens and make these available for diagnostic testing and evaluation in assays for cell-mediated immunity.

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $128,000

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Soluble Human Tumor (CEA) Specific Antigens

Principal Investigator: Dr. Ralph Reisfeld
Name/Address Scripps Clinic and Research Foundation
Performing La Jolla, California 92037
Organization:

Contract Number: NO1-CP-43277
Starting Date: 1/1/74 Expiration Date: 12/31/76

Goal: To isolate and characterize soluble human tumor (carcinoembryonic antigen) specific antigens.

Approach: Isolation of CEA by mild extraction procedures, purification and characterization of CEA, production of anti-CEA antisera and development of an isotope release cytotoxicity assay with CEA positive cell lines.

Progress: Significant proteolytic activity has been shown in tumor hemogenates continuing 1 M perchloric acid showing that early steps in the classical purification procedure may introduce heterogeneity into the CEA fraction at steps other than the lyophilization procedure. With the aid of immunoabsorbents, CEA was isolated from various human sera including that of lung and colon cancer patients. It appears that immunobsorbent bound CEA injected into rabbits produces antibody which reacts specifically with colon or lung cancer CEA with no cross-reacting activities.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $71,909

CONTRACT RESEARCH SUMMARY

Title: Detection of Circulating Antigen-Antibody Complexes in Cancer

Principal Investigator: Dr. Argyrios N. Theofilopoulos
Name/Address Scripps Clinic and Research Foundation
Performing 476 Prospect Street
Organization: La Jolla, California 92037

Contract Number: NO1-CB-53952
Starting Date: 5/16/75 Expiration Date: 5/15/76

Goal: To determine whether detection of circulating complexes could aid in the diagnosis of cancer.

Approach: Use of the Raji cell's ability to bind immune complexes efficiently for detection and quantitation of immune complexes in cancer patients' sera; to study the relationships of the complexes in the sera to pathogenesis, prognosis and diagnosis of their disease.

Progress: A sensitive, simple procedure for detection and quantitation of soluble complement-fixing immune complexes has been developed using complement receptors on Raji cells. Using this assay the contractor detected immune complexes in sera of patients in various disease stages, malignancy included. Over one thousand sera from cancer patients have been screened. While most sera are still coded, preliminary findings of uncoded sera indicate approximately 4% of cancer patients have circulating immune complexes. Sera of lymphoid tumor patients appear to contain higher levels of complexes than sera from solid tumor patients. A survey of serial bleedings of a few melanoma patients showed good correlations between presence of immune complexes and clinical status.

Significance for Cancer Research (NCP Objective 3 Approach 4)
Identification and study of tumor associated antigens and antibodies are an important part of the Baseline Information Flow of the Tumor Immunology Program.

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $89,992

CONTRACT RESEARCH SUMMARY

Title: Isolation and Tissue Culture of Human Tumor Cells

Principal Investigator: Jorgen E. Fogh, M.D.
Name/Address Performing Organization: Sloan-Kettering Institute for Cancer Research
145 Boston Post Road
Rye, New York 10580

Contract Number: NO1-CB-43854
Starting Date: 8/08/73 Expiration Date: 8/7/76

Goal: To provide human tumor materials needed for the performance of assays for cell-mediated immunity.

Approach: Tumor specimens from patients having the more common tumors, such as carcinomas of the colon, stomach, lung, breast, prostate, and bladder, will be obtained from local sources and utilized for the development of techniques suitable for the isolation of viable tumor cells in good yield separated from contaminating normal cells. Tissue culture techniques which favor the maintenance of high viability of tumor cells will be developed. Cells maintained in such culture will be characterized by morphology and as many other techniques as possible to document their tumor origin. Techniques will be developed for the slow freezing and retrieval of viable cells suitable for growth in tissue culture or for immediate use in immunologic assays.

Progress: Dr. Fogh has collected an extensive number of early cultures of human cancer cells and established tumor cell lines. He maintains an updated catalog of these lines and is proceeding with detailed characterization of the lines. His lines serve as an excellent resource to the scientific community and demand is increasing.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Studies of cell-mediated immunity towards specific tumors as well as a general phenomenon in cancer patients is an urgently needed area of development in tumor immunology.

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date: 10/31/73
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $208,054

CONTRACT RESEARCH SUMMARY

Title: Role of Antibody Dependent Cell-Mediated Cytotoxicity in Tumor Immunity

Principal Investigator: Dr. Osias Stutman
Name/Address Sloan-Kettering Institute
Performing 425 East 68th Street
Organization: New York, New York 10021

Contract Number: NO1-CB-53894
Starting Date: 5/01/75 Expiration Date: 4/30/76

Goal: Determine the importance of cytotoxicity of syngeneic tumor cells by antibody-dependent cell-mediated mechanisms in host resistance to tumor growth, and whether detection of antibodies mediated this effect would be useful for detection of tumors for monitoring of tumor growth.

Approach: Study the role of antibody-dependent cell-mediated cytotoxicity in tumor immunity in a mouse system using spontaneous mammary adenocarcinoma.

Progress: A reliable assay for antibody-dependent cell cytotoxicity (ADCC) using a syngeneic mouse system was developed. The antibody has activity in IgG subclasses and not in IgA or IgM and it is thymus dependent in development. (The C3H antibody is produced by hyperimmunization with mammary tumor cells.) Adherent C3H mammary tumors prelabeled with ^{3}H proline are target cells. Effector cells are from normal C3H or C3HF donors.

Significance for Cancer Research (NCP Objective 6 Approach 4 and Objective 3 Approach 4)
Studies of cells involved in the immune destruction tumors are an important part of Baseline Information Flow in the Tumor Immunology Program.

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $57,382

CONTRACT RESEARCH SUMMARY

Title: Endoglycosidases Capable of Releasing Oligosaccharides from Glycoproteins

Principal Investigator: Dr. Om Bahl
Name/Address State University of New York
Performing Buffalo, New York 14214
Organization:

Contract Number: NO1-CB-43989
Starting Date: 5/01/74 Expiration Date: 4/30/76

Goal: To define the nature of the antigenic determinants of carcinoembryonic antigen and other cellular glycoproteins.

Approach: Develop enzymatic methods for obtaining oligosaccharides with serological activity. Purify and characterize endoglycosidases that will act on CEA, blood group glycoproteins or other macromolecules containing carbohydrate antigenic determinants.

Progress: Various substrates such α_1-acid glycoprotein, fetuin, and porcine submaxillary mucin and their glycopeptides were prepared. A neuraminidase inhibitor, N-(p -aminophenyl)-oxamic acid was synthesized. The assay procedure for endoglycosidases was further developed and optimal growth conditions for various microorganisms were worked out. A number of these microorganisms were examined for endoglycosidase activity.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $53,704

CONTRACT RESEARCH SUMMARY

Title: Detection of Circulating Antigen-Antibody Complexes in Cancer

Principal Investigator: Dr. Ralph E. Schrohenloher
Name/Address University of Alabama
Performing Birmingham, Alabama 35294
Organization:

Contract Number: N01-CB-53893
Starting Date: 5/01/75 Expiration Date: 4/30/76

Goal: To determine whether detection of circulating complexes could aid in the diagnosis of cancer.

Approach: Conduct a search for immune complexes by developing sensitive quantitative methods; characterize isolated complexes and screen sera for complexes from a broad cross section of patients.

Progress: Development of sensitive quantitative methods for detection of immune complexes where antigen is now known has been initiated. The three approaches being used are bases on reaction with the Clq component of complement, human monoclonal rheumatoid factor and the Raji line of lymphoblastoid cells.

Significance for Cancer Research (NCP Objective 3 Approach 4)
Identification and study of tumor associated antigens and antibodies are an important part of the Baseline Information Flow of the Tumor Immunology Program.

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $78,733

CONTRACT RESEARCH SUMMARY

Title: Development of Immunodiagnostic Tests for Cancer

Principal Investigator: Dr. Henry Weimer
Name/Address Performing Organization: University of California at Los Angeles
Los Angeles, California 90024

Contract Number: NO1-CB-43860
Starting Date: 9/01/73 Expiration Date: 8/31/76

Goal: To identify new tumor associated antigens in carcinomas of the colon, stomach, lung, breast, prostate, and bladder which might be useful for immunodiagnosis.

Approach: Extract antigens from these carcinomas and from comparable normal tissues and from fetal tissues and prepare heterologous antisera against each of the antigens. Antigens shall be purified and antisera rendered specific for tumor associated antigens. Radioimmunoassays shall be developed for the detection of each antigen in serum or urine.

Progress: Large numbers of tumor, normal and fetal tissue specimens have been collected and a serum bank has been started in anticipation of development of a "screening" assay for tumor associated antigens. Crude membrane preparations have been isolated from solid lung tissue and various methods of solubilizing membrane fractions are being evaluated. Antisera to cell membrane fractions have been raised in rabbits and tested for immunologic reactivity. Lung cell membrane fractions have also had extensive chemical and electrophoretic analysis. Fetal tissues have been fractionated and fractions have been analyzed for cell surface antigens, insulin binding and various enzymes.

Significance for Cancer Research (NCP Objective 5 Approach 4)
New and more specific immunodiagnostic tests for the common types of human cancer are needed.

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date: 7/23/75
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $157,828

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Human Peripheral Blood Monocytes

Principal Investigator:
Name/Address
Performing
Organization:

Dr. David Talmage
Webb-Waring Lung Institute and
University of Colorado Medical Center
4200 East Ninth Avenue
Denver, Colorado 80220

Contract Number: NO1-CB-53900
Starting Date: 5/16/75 Expiration Date: 5/15/76

Goal: To develop procedures for more effective and reproducible fractionation of monocytes from human peripheral blood.

Approach: Isolation of human peripheral blood monocytes from normals and cancer patients using a modified design of counterflow centrifugation and test these cells for functional activity and markers.

Progress: In this initial contract period a procedure for isolating monocytes from human peripheral blood by counterflow centrifugation was established. During the procedure, the lymphocytes and monocytes are separated from one another with comparatively little contamination. Monocytes, varying considerably in maturity, are contaminated by occasional plasma cells and other types. Between 35 and 44 percent of all mononuclear cells obtained from the ficoll-hypague preliminary separation are able to phagocytose and are peroxidase positive. Separated fractions have been obtained with from 0 to 95 percent positivity for both phagocytosis and peroxidase. There has been some problem with monocytes sticking to centrifuge tubing, especially when made to phagocytose prior to separation..

Significance for Cancer Research (NCP Objective 5 Approach 7)
Improve methods and programs for cancer detection and screening

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $48,280

CONTRACT RESEARCH SUMMARY

Title: Animal Models for Circulating Tumor Associated Antigens for Diagnosis of Carcinoma

Principal Investigator: Dr. James W. Osborne
Name/Address University of Iowa
Performing Iowa City, Iowa 52242
Organization:

Contract Number: NO1-CB-43891
Starting Date: 6/01/74 Expiration Date: 5/31/76

Goal: Perform studies of circulating tumor associated antigens in animal carcinomas that would serve as a model for common human cancers and that are potentially useful in developing immunodiagnostic tests for cancer.

Approach: Study antigens associated with radiation induced intestinal tumors in mice. Demonstrate and isolate circulating tumor associated antigens and design specific tests for quantitating the antigen. Study the relationship of antigen appearance to pre-malignant lesions and determine the levels of antigen in relation to tumor size.

Progress: A tumor-associated protein was found in tissue derived from an x-irradiation induced adenocarcinoma in the small bowel of the rat. It shared common antigenic determinants both with a rat fetal protein and a perchloric-acid-soluble protein isolated from the serum of a tumor bearing rat. The immunological activity of the tumor associated protein was acid labile and alkali stabile. Endo-and exopeptidases eliminated discernible immunological activity but nucleotidases and neuraminidase did not affect the activity. Preliminary results from binding studies with ^{125}I-labeled antigenically active protein indicate that a radioimmunoassay may permit concentration measurements of the protein. Further efforts were directed at improvement of the reproducibility of the immunoradiometric assay.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $90,219

CONTRACT RESEARCH SUMMARY

Title: Experimental Systems for Carcinoembryonic Antigen

Principal Investigator: Dr. David M. Goldenberg
Name/Address University of Kentucky
Performing Lexington, Kentucky
Organization:

Contract Number: NO1-CB-33869
Starting Date: 6/30/73 Expiration Date: 11/30/75

Goal: To produce carcinoembryonic antigen (CEA) which will then be provided to other investigators for analysis of its structure and to provide cell lines which produce CEA for use in studies of cellular hypersensitivity in patients.

Approach: Will develop two human colon tumor lines maintained in hamster cheek pouches for the purpose of providing carcinoembryonic antigens in quantity to other investigators for analysis of the structure of the material. Will provide cell lines derived from the tumors carried in hamster cheek pouches for the purpose of studying cellular hypersensitivity in patients with various carcinomas.

Progress: Human tumor material from GW-39 tumors has been supplied to seven laboratories. Species specificity and carcinomebryonic antigen content of numerous GW-39 tumor cell lines have been determined as well as their malignant potential in hamsters. Various other cell lines have been propagated and characterized. A high molecular weight CEA has been isolated from GW-39 tumor tissue and chemical characterization has been done on it. The identity of GW-39 CEA to reference human CEA has been established via radioimmunoassay and immunodiffusion.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Structural analysis of CEA from various sources will improve understanding of its relationship to tumor origin and size.

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $12,500

CONTRACT RESEARCH SUMMARY

Title: Procurement of Tumor Specimens and Serial Serum Specimens from Cancer Patients

Principal Investigator:
Name/Address
Performing
Organization:

Eugene B. Rosenberg, M.D.
University of Miami
Coral Gables, Florida 33124

Contract Number: NO1-CB-43861
Starting Date: 8/15/73 Expiration Date: 8/31/75

Goal: Collection of serum specimens from patients with malignant diseases over a period of time to be used for the development and evaluation of potential immunodiagnostic tests for cancer.

Approach: Serum and plasma specimens will be obtained from patients with carcinomas of the colon, lung, breast, prostate, bladder, and other malignant diseases and from patients with benign diseases. A minimum of 25 patients per year of each category will be obtained. Specimens will be obtained before any therapy and at weekly intervals for one month and every two to three months thereafter.

Surgical specimens of the above tumors to the extent of a minimum of 25 tumors of each type per year will be obtained under sterile conditions and in conjunction with normal control tissues from the same organ sites. Serum, EDTA, plasma, and heparinized bloood from some patients may also be required. In addition, a certain amount of dissection of tumor tissue and preparation of single cell suspensions, with appropriate packaging for shipping fresh and frozen specimens will be done.

Progress: A total of 260 tumors and 1300 blood and serum samples have been supplied to investigators. Bladder, skin, breast, colon, kidney and liver are among the tumors procured. Serial serum specimens have been collected on cancer patients prior to, during and after treatment.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Readily available collection of sera obtained from patients during the course of therapy for their cancers are necessary for prompt evaluation of promising new immunodiagnostic techniques.

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $155,700

CONTRACT RESEARCH SUMMARY

Title: Collection of Sera from Populations with High Cancer Risk

Principal Investigator: John S. Narjarian, M.D.
Name/Address University of Minnesota
Performing Minneapolis, Minnesota 55455
Organization:

Contract Number: NO1-CB-33915
Starting Date: 6/30/73 Expiration Date: 4/30/76

Goal: To collect serial serum specimens from populations at high risk of developing malignant disease for the purpose of developing and evaluating immunodiagnostic screening tests for human cancer.

Approach: Stable populations at high risk of developing the more common human cancers, such as colon, lung, breast, prostate, or bladder, will be followed on an annual basis. This will include clinical evaluation and serum collection. The sera will be banked and records will be kept on the patients so that stored sera can be correlated with development of any particular cancer.

Progress: At UMCDC 3509 asymptomatic adults in high cancer risk age groups have been examined for cancer in the past year. Complete histories and physicals plus a special "allergy" history were done. Serum was collected at the time of the examination and sent coded to Mayo Clinic. Of these patients, 32 have developed histologically proven malignancies but overall incidence is expected to be higher since final information on diagnoses takes over a year to complete.

Significance for Cancer Research (NCP Objective 5 Approach 1)
Early detection of cancer by means of appropriate diagnostic testing would permit treatment of cancer at the most favorable time.

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $78,255

CONTRACT RESEARCH SUMMARY

Title: Assays for Immune Depression in Cancer Patients

Principal Investigator: Edmond J. Yumis, M.D.
Name/Address University of Minnesota
Performing Minneapolis, Minnesota 55455
Organization:

Contract Number: NO1-CB-43853
Starting Date: 3/1/73 Expiration Date: 9/30/75

Goal: To develop quantitative assays for the depression of various aspects of the immune response in human cancer patients and for following the clinical course of cancer patients.

Approach: Various parameters of the immune response will be evaluated in patients having carcinomas of the lung, breast, colon, prostate and bladder. These assays will be potentially applicable to large-scale use and will have been previously shown to be depressed in some cancer patients or to be depressed in animals with experimental tumors.

Progress: Patients with basal cell cancer and arious carcinomas were tested for the percent of T and B cells in peripheral blood, and for the ability of their lymphocytes to be stimulated by mitogens, antigens and allogeneic leukocytes. It was found that the cancer patients as a group had significant depression in stimulation by some mitogen doses and by some antigens. The total T and B cells in the peripheral blood of cancer patients was generally the same as that of the normal controls.

Significance for Cancer Research (NCP Objective 5 Approach 1)
Assays for evaluation of the immune responsiveness of given individuals may prove to be highly important in understanding cancer patients' susceptibility to their tumors

Profject Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $14,072

CONTRACT RESEARCH SUMMARY

Title: Immunofluorescent Detection of Antibodies Against Autologous Human Tumor Cells

Principal Investigator: Dr. George J. Friou
Name/Address University of Southern California
Performing 2025 Zonal Avenue
Organization: Los Angeles, California 90033

Contract Number: NO1-CB-43881
Starting Date: 6/1/74 Expiration Date: 5/31/76

Goal: To identify specific antibodies in the sera of patients with carcinoma of the lung, breast, and colon.

Approach: Sera from cancer patients and from controls will be tested against autologous tumor cells by immunofluorescence assays with viable and fixed tumor cells. Sera for testing will be obtained before and at appropriate intervals after therapy. Specificity of observed reactions will be determined.

Progress: Biopsy materials and blood samples from controls and tumor patients with cancer of the breast, colon and lung were collected. Tumor tissue, normal tissue or sera were all stored. Indirect immunofluorescent testing was done for serum antibodies against cytoplasmic and membrane antigens of tumor cells, using autologous and allogeneic tissue. Absorption of positive sera with homogenates of autologous tumors abolished reactivity. A partial reduction in antibody titer was observed when positive sera were absorbed with allogenic tumors as well as with tissues from fibroadenoma and fibrocystic disease. Absorption with various normal rat or human organ homogenates did not effect antibody titer. Using monospecific antiserum, the antibody to cytoplasmic antigens was shown to belong to the IgGt class of immunoglobulins.

Significance for Cancer Research (NCP Objective 5 Approach 2)

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $79,923

CONTRACT RESEARCH SUMMARY

Title: New In Vitro Techniques to Evaluate Cell Mediated Immunity to Intact Tumor Cells

Principal Investigator: Dr. John T. Harrington
Name/Address Performing Organization: University of Texas Medical School
San Antonio, Texas 78284

Contract Number: N01-CB-43893
Starting Date: 6/1/74 Expiration Date: 10/31/75

Goal: To develop new in vitro techniques or substantial modifications of existing techniques for the evaluation of cell-mediated immunity to whole tumor cells.

Approach: Develop Agarose droplet modification of migration inhibition assay for application to detection of immunity to human tumor antigens.

Progress: An agarose droplet micromethod has been developed, using EL-4 mouse lymphoma model tumor and the assay has been validated by several parameters of MIF response. Partial biochemical characterization has been performed on a lymphokine showing inhibitory activity by this method of measuring MIF synthesis. Optimal cryopreservation methods have been developed by testing the functional activity of EL-4 lymphoma cells and hyperimmune mouse spleen cells before and after freezing. Optimal conditions have been established for preparing human peripheral blood leukocytes using plasma-gel sedimentation and Hypaque-Ficoll gradient leukocyte fractionation. Techniques have also been developed for processing, culturing and cryopreserving tumor cells from human breast carcinomas.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $75,609

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Human Peripheral Blood Mononuclear Cells

Principal Investigator: Dr. Hans Wigzell
Name/Address University of Uppsala
Performing Uppsala, Sweden
Organization:

Contract Number: NO1-CB-43883
Starting Date: 6/25/74 Expiration Date: 6/24/76

Goal: Development of procedures for more effective and reproducible fractionation of mononuclear cells from human peripheral blood.

Approach: Peripheral blood from normal individuals and from patients with carcinomas will be studied. The following cell types will be isolated in suspension with high yields, purity, and viability: T lymphocytes, B lymphocytes, and monocytes. The isolated cells will be characterized for viability, morphology, retention of membrane markers, and retention of immunological functions.

Progress: Human blood nononuclear cells were separated into subgroups using a variety of techniques. The specificity of the Fc receptor of the human lymphocytes involved in ADCC has been studied in detail using purified IgG of different species and subclasses. No species specificity has been found. Inhibitory capacity of human IgG subclasses was found to parallel their binding capacity to Fc receptors. Earlier reported differences in binding to human Fc receptors according to IgG subclass could be shown largely due to differences in the "natural" aggregability of the different IgG subclasses. Using methods accounting for unit surface areas no differences could be shown between the four IgG subclasses. Performances of purified T and T-depleted, B-enriched lymphocyte populations as cytotoxic effectors against antibody-coated erythrocytes or tumor cells indicated initial lack of activity of purified T cells irrespective of whether IgM or IgG antibodies were used to induce ADCC.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $33,500

CONTRACT RESEARCH SUMMARY

Title: Antibodies to Human Organ or Tissue Associated Antigens

Principal Investigator: Dr. Jen-Fu Chiu
Name/Address Performing Organization: Vanderbilt University School of Medicine
Nashville, Tennessee 37232

Contract Number: NO1-CB-53896
Starting Date: 6/16/1975 Expiration Date: 6/15/76

Goal: To detect antibodies to human organ or tissue associated antigens to use in study of antigenic profile of normal and neoplastic cells both from biopsy and tissue culture.

Approach: Obtain specific antibodies to chromosomal nonhistine proteins associated with homologous DNA, and measure in tumor cells the quantitative decrease or loss of normal antigens and increase of tumor specific antigens. Distribution and expression of these antigens will be studied in relation to disease type, stage of disease, and prognosis.

Progress: Specific antisera was made against human lung and breast carcinomas. Testing results indicate that specific antibodies can be produced not only against various tissues but also against individual types of malignant tumors and is at variance with their previous experiments with transplantable animal tumors. Localization of tissue-specific nuclear nonhistone protein-DNA antigens was attempted with the horse radish peroxidase bridge technique and antigen clearly localized in the nucleus of the cells. Studies proceed to determine the localization specificity of antisera against human lung carcinoma as compared with normal tissue.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit: 4/25/75
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $86,282

CONTRACT RESEARCH SUMMARY

Title: Development of Macrophage Electrophoretic Mobility Assay for Malignant Disease

Principal Investigator:
Name/Address
Performing
Organization:

Dr. John A. Pritchard
Velindre Hospital
South Wales and Monmouthshire
Radiotherapy Service
Whitchurch, Cardiff,
Wales CF4 7XL

Contract Number: NO1-CB-53956
Starting Date: 6/29/75
Expiration Date: 6/29/76

Goal: To further evaluate the macrophage electrophoretic mobility (MEM) test, to determine its possible clinical applications, and to either standardize the current assay or develop an improved method for detection of reactivity.

Approach: Use a biochemical approach to study the nature of the effector compound released in cancer-sensitized lymphocytes, plus clinical appraisal to further develop the MEM and a modified MEM test.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $38,000

CONTRACT RESEARCH SUMMARY

Title: Detection of Circulating Antigen-Antibody Complexes in Cancer

Principal Investigator: Dr. Dieter Burger
Name/Address Performing Organization: Washington State University
Pullman, Washington 99163

Contract Number: NO1-CB-63990
Starting Date: 9/01/75 Expiration Date: 8/31/76

Goal: To determine whether detection of circulating complexes could aid in the diagnosis of cancer.

Approach: Purification of Antigen antibody complexes in either a feline leukemia or rat bladder tumor system and evaluation of these complexes. Possible correlation between quantity of complexed antibody and progression of the tumor shall be investigated.

Progress: New Contract

Significance for Cancer Research (NCP Objective 3 Approach 4)
Identification and study of tumor associated antigens and antibodies are an important part of the Baseline Information Flow of the Tumor Immunology Program.

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $34,757

CONTRACT RESEARCH SUMMARY

Title: Search for New Human Tumor Associated Antigens in Carcinoma of the Lung

Principal Investigator: Dr. Robert W. Veltri
Name/Address Performing Organization: West Virginia University
Morgantown, West Virginia 26505

Contract Number: NO1-CB-43890
Starting Date: 5/16/74 Expiration Date: 5/15/76

Goal: Search for new tumor associated antigens in carcinoma of the lung, which may be more useful in immunodiagnosis than currently available markers.

Approach: Identify by immunological methods antigens which may be useful in detection of carcinoma of the lung. Employ these methods in screening both biopsy specimens and cell cultures of lung tumors, characterize detected antigens and determine distribution of any new antigen or related antibody in patients with cancer, benign disease, and in normal controls.

Progress: Progress during the past year has been as projected in that three proteins, called α,β and γ, have been isolated from at least two lung tumors. These were identified by rabbit antiserums developed to extracts from several other lung tumors. α seems to be a serum protein, but β and γ appear to be lung-specific TAA. Collaborative studies with purified α and β suggest that neither is αFP, CEA or β-2 Microglobulin.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Dr. Frederic Mushinski
Program: Immunodiagnosis Site Visit Date: 3/15/74
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $114,214

CONTRACT RESEARCH SUMMARY

Title: Early Detection of Antibodies to Carcinomas in Experimental Animal Models

Principal Investigator: Dr. Lionel Manson
Name/Address The Wistar Institute
Performing 36th Street at Spruce
Organization: Philadelphia, Pennsylvania 19104

Contract Number: NO1-CB-43882
Starting Date: 6/1/74 Expiration Date: 5/31/76

Goal: Development of experimental animal systems for the early detection of antibody response to tumor associated antigens, as model for problem of antibody detection in patients with carcinomas and other malignant diseases.

Approach: Studies will be performed with primary, autochthonous carcinomas or with carcinomas in early transplant, in inbred strains of mice. A sensitive antiglobulin assay will be used to determine antibody levels.

Progress: Studies with spontaneous mammary adenocarcinoma A-10 have been continued. No humoral response has been demonstrated during growth of the A-10 tumor line from small inocula either subcutaneously or intraperitoneally in A/J or A/He mice. An antibody population has been found in sera of a variety of normal mouse strains in addition to A/J and A/He, and in sera of A/J tumor-bearers. The antibody population has also been eluted from ascitic-growing A-10 cells. All these sources of antibody appear to have the identical property of non-specific sticking to a variety of cell lines. No specific A-10 antibody has been found in the syngeneic host. The stage 1 radio-immunoassay has been modified so it can readily be used with cell lines that attach to plastic sera and spontaneous mammary adenocarcinomas have been accumulated form C_3H mice.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Mrs. Judith Whalen
Program: Immunodiagnosis Site Visit Date: 2/26/74,2/27/76
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $82,000

CONTRACT RESEARCH SUMMARY

Title: Biochemistry and Diagnostic Use of Human Tumor Antigens

Principal Investigator: Dr. Vincent Marchesi
Name/Address Yale University
Performing New Haven, Connecticut
Organization:

Contract Number: NO1-CB-43859
Starting Date: 8/8/73 Expiration Date: 11/07/76

Goal: To isolate and purify glycoprotein antigens from human colonic tumors.

Approach: The contract will use various extraction methods to isolate glycoproteins from human colonic tumors and prepare specific antisera to each of the isolated antigens. The specificity of these antigens will be compared with carcinoembryonic antigen prepared by usual procedures. Glycoproteins extracted from primary colon tumors, metastatic tumors, serum, and from embryonic tissues will be examined.

Progress: A lectin-sepharose affinity chromatography system with high capacity has been developed which will enable the contractor to develop large scale isolation procedures. These affinity systems, are very effective for the binding of the CEA-like material as isolated from colonic carcinoma patients. Conditions for the adsorption of glycoproteins and CEA-like materials to the columns and for removal of the glycoproteins have been determined. Work has been done on refining conditions for the isolation of membrane glycoproteins which will minimize degradation of sugar, protein and antigenic moieties. All but one of the Aldonolactone derivatives needed to inhibit the glycosidases active against the linkages the contractor expects to find in membrane glycoproteins have been synthesized.

Significance for Cancer Research (NCP Objective 5 Approach 4)
New methods for extraction of glycoprotein antigens from human colonic tumors may provide reagents with better specificity for immunodiagnosis than CEA.

Project Officer: J. Frederic Mushinski, M.D.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $39,960

CONTRACT RESEARCH SUMMARY

Title: Intrapleural BCG after Primary Surgery for Lung Cancer

Principal Investigator:
Name/Address
Performing
Organization:

Martin F. McKneally, M.D.
Albany Medical College
Division of Thoracic Surgery
ME-622
Albany, New York 12208

Contract Number: NO1-CB-53940
Starting Date: 6/1/75 Expiration Date: 5/31/76

Goal: Evaluate the therapeutic efficacy of intrapleural BCG with or without tumor antigen preparations in lung cancer.

Approach: Basic methodologies will be developed in animal models and applied to humans under appropriate designed clinical protocols.

Progress: The hazards associated with this approach have been minimized by the use of a single limited dose of microorganisms, isoniazid, and careful patient monitoring. A preliminary evaluation of the clinical protocol suggests that intrapleural BCG in patients with stage I lung cancer may be effective in improving survival.

Significance for Cancer Research (NCP Objective 6 Approach 4)
Regional immunotherapy has provided the most promising results in melanoma and in animal models. This project is a logical extension of such work.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: Estimated $60,000

CONTRACT RESEARCH SUMMARY

Title: Augmentation of Tumor-Specific Immunity with Ribonucleic Acid (RNA)

Principal Investigator:
Name/Address
Performing
Organization:

Peter J. Deckers, M.D.
Department of Surgery
Boston University School of Medicine
80 East Concord Street
Boston, Massachusetts 02118

Contract Number: N01-CB-43949
Starting Date: 6/01/75 Expiration Date: 5/31/76

Goal: Development of a reliable in vivo immunotherapy model with immune RNA in laboratory animals.

Approach: Analysis of the variables associated with optimal extraction and administration of RNA will be studied in small animal models for evaluation of its immunotherapeutic effect.

Progress: The investigator has experienced considerable difficulty reproducing the results of others. Every effort is being made to clarify the nature of the negative data and to reconcile any technical problems that might be responsible for the discrepancies.

Significance for Cancer Research (NCP Objective 2,4 Approach 1,2,3,4)
Subcellular fractions such as immune RNA may be relatively nontoxic and possibly highly specific approaches to cancer immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: Estimated $50,000

CONTRACT RESEARCH SUMMARY

Title: Simple Light Microscope Test for B and T Cells on Dried Blood Films

Principal Investigator:
Name/Address
Performing
Organization:

William J. Dreyer, Ph.D.
California Institute of Technology
c/o Jet Propulsion Laboratory
4800 Oak Grove Boulevard
Pasadena, California 91109

Contract Number: NO1-CB-43875 Expiration Date: 11/30/74

Goal: Development of a simple histochemical assay for B and T cells.

Approach: Utilizing antibodies specific for mouse B and T cells, the investigator will develop immunolatex reagents specifically for use in conjunction with standard Romanovsky stains. Once the model system has been developed in mice, a human antiserum will be utilized for the development of readily applicable simplified B and T cell assay.

Progress: The contractor has successfully labelled murine B-cells and thymocytes with latex particles coated with anti-sera as evaluated by scanning electron and light microscopy. In addition, information on the necessary design of non-aggregating and bio-compatible latex particles for use in light microscopy has been developed. This project was completed November, 1975.

Significance for Cancer Research (NCP Objective 4,6 Approach 1,2,4)
Widespread availability of simplified B and T cell assays would be extremely valuable for monitoring immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Hybrid Tumor Cells: An Immunotherapeutic Agent

Principal Investigator: Robertson Parkman, M.D.
Name/Address Performing Organization: Children's Hospital Medical Center
300 Longwood Avenue
Boston, Massachusetts 02115

Contract Number: N01-CB-43876
Starting Date: 05/16/74 Expiration Date: 5/15/75

Goal: Evaluate hybrid cells formed by fusion of tumors with non-tumor cells as immunotherapeutic agents.

Approach: Syngeneic animal systems will be used to evaluate the capacity of cells formed by fusion of tumor cells with fibroblasts to alter morbidity and mortality of tumor-bearing animals. In vitro comparison of immune response to hybrid tumor cells, irradiated tumor cells and live tumor cells in normal, tumor-bearing, and "carrier" immunized animals will be evaluated.

Progress: The contractor has achieved fusion of C_3H fibroblasts with a methylcholanthrene induced fibrosarcoma after deriving drug-resistant mutants of the tumor. Similar fusion has been achieved with B-16 melanoma. These hybrid tumor cells have been shown to be capable of immunizing host animals against subsequent lethal tumor challenge.

Significance for Cancer Research (NCP Objective 2,6 Approach 1,4)
This project will explore a promising new direction in cancer therapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Evaluation of C. Parvum in Advanced Cancer of the Breast and Lung

Principal Investigator: John Fahey, M.D.
Name/Address Performing Organization: Prof. & Chairman, Dept. of Medical Microbiology & Immunology
Center for the Health Sciences
Los Angeles, California 90024

Contract Number: NO1-CB-53939
Starting Date: 6/16/75 Expiration Date: 6/15/76

Goal: The usefulness of Corynebacterium Parvum and BCG will be evaluated as adjuncts to chemotherapy in the treatment of disseminated breast cancer and lung cancer.

Approach: See goal

Progress: Logistics and feasibility aspects of the clinical trials have been worked out, and patients are being entered into the protocols at an acceptable rate during the first contracting period.

Significance for Cancer Research (NCP Objective 6 Approch 4)
Cancer of the breast and lung are two of the most common varieties of cancer. Evaluation of immunotherapy in these tumors has a high priority.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: Est. $110,000

CONTRACT RESEARCH SUMMARY

Title: Immunotherapeutical Trials with Human Tumors

Principal Investigator:
Name/Address
Performing
Organization:

Dr. Karl Erik Hellstrom
Fred Hutchison Cancer Research Center
Seattle, Washington 98195

Contract Number: NO1-CB-64018
Starting Date: 10/30/75 Expiration Date: 09/30/76

Goal: To evaluate the use of "deblocking" serum as well as immunization with autochthonous tumor cells as possible therapeutic approaches in the treatment of malignant melanoma. BCG may also be used.

Approach: Preliminary studies will be carried out to assure that neither of the immunotherapeutic maneuvers appears to have toxic effects on recipients. After this has been established, a double-blind immunotherapy trial will be established wherein patients will be assessed to determine whether their serum contains blocking antibodies in the colony inhibition assay. Patients with blocking antibodies will be randomized into a test group which will receive deblocking plasma and a control group which will receive normal plasma. Patients whose serum does not contain blocking factor will be randomized into a treatment group that will receive a course of immunotherapy involving active immunization with intradermal inoculations of tumor cells mixed with living BCG, and a control group which will receive no additional therapy. A variety of laboratory investigations will be carried out on these patients.

Progress: This study has accrued more than 60 patients. There has, as yet, been no striking difference between the experimental and control groups and the code relative to which patients are receiving normal plasma and which are receiving unblocking plasma has not yet been broken.

Significance for Cancer Research (NCP Objective 6 Approach 4)
This contract will provide indepth laboratory analyses of patients' immune responses during immunotherapy and coordination of these responses with clinical course

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: 10/4/74
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $173,117

CONTRACT RESEARCH SUMMARY

Title: Preclinical Studies on Tumor Protective Activity of MER

Principal Investigator: David W. Weiss
Name/Address Professor and Chairman, Department of Immunology
Performing Lautenberg Center for General & Tumor Immunology
Organization: Hebrew University - Hadassah Medical School
Jerusalem, Israel

Contract Number: NO1-CB-02208
Starting Date: 8/29/75 Expiration Date: 2/28/77

Goal: Study immunotherapeutic effects of MER and its mechanism of action.

Approach: Evaluation of MER as a therapeutic agent combined with irradiation and/or chemotherapy in prevention of metastatic tumors in mice and guinea pig models, including optimization of dose and schedule of MER. These studies will also be applied to spontaneous mammary tumors and to both mice and guinea pigs subjected to chemical oncogenic agents, early in the induction of tumors and after palpable tumors have arisen. The mode of action of MER will be assayed with a number of different in vitro techniques for cell-mediated and humoral immunity, some of which will utilize tumor cells and specific targets. In addition, the possibility that MER, BCG organisms, certain other subcellular components of BCG and a large variety of taxonomically unrelated bacterial pathogens may share common antigens with a number of different neoplastic cell types is being investigated.

Progress: MER with either irradiation or chemotherapy represents the form of treatment most likely to be effective against solid tumors in the animal models used. Preliminary models for study of the effects of MER alone on pulmonary metastases in MTV mammary carcinomas had been initiated and early evidence that MER plus specific immunization with tumor cells and X-ray therapy could be a valuable form of therapy is being accumulated. Studies in immunoprophylaxis against mice mammary tumors have helped define parameters of MER administration which would not induce enhancement. MER has been demonstrated to be effective as an immunoprophylactic agent in Line 10 hepatoma of Strain 2 guinea pigs; therapy experiments have been initiated. A number of studies directed towards evaluation of the mode of action of MER are in progress and new techniques for these studies are being developed.

Significance for Cancer Research (NCP Objective 6 Approach 1,4)
Development of preclinical models for immunotherapy and evaluation of mechanism of action in such models is the basis for rational approaches to human immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: 9/2/74
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $179,098 (Terminal period - 18 months)

CONTRACT RESEARCH SUMMARY

Title: Preparation and Distribution of Rabbit Serum Complement

Principal Investigator: Marianna Cherry, Ph.D.
Name/Address The Jackson Laboratory
Performing Box 258
Organization: Otter Creek Road
Bar Harbor, ME 04609

Contract Number: N01-CB-53870
Starting Date: 06/16/75 Expiration Date: 6/15/76

Goal: Find rabbit complement sources that do not show nonspecific cytotoxicity.

Approach: Inbred rabbits will be evaluated for their complement activity.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: Estimated $110,000

CONTRACT RESEARCH SUMMARY

Title: The Immunological Stimulant Properties of Amphotericin B

Principal Investigator: J. Russell Little, M.D.
Name/Address Jewish Hospital of St. Louis
Performing 218 South Kingshighway
Organization: St. Louis, Missouri 63110

Contract Number: NO1-CB-43872
Starting Date: 03/01/76 Expiration Date: 02/28/77

Goal: To evaluate immunologic responses in animal models receiving Amphotericin B (AmB).

Approach: B and T cell responses will be evaluated in animals receiving AmB following immunizations with soluble hapten protein conjugates. Other aspects of the humoral and cellular immune response will also be evaluated in relation to SRBC administration. The rejection time of histo-incompatible skin grafts and GVH will also be evaluated in reaction to AmB. Macrophage responses and AmB effects on the immunosuppressed animal will also be evaluated.

Progress: The investigators have demonstrated an immuno-stimulant effect in BALB-C mice for humoral immune responses; macrophage activation has been shown by in vitro and in vivo studies; a toxicity of Amphotericin B for murine thymocytes has been demonstrated; and an increase in the serum level of colony-stimulating factor after administration of Amphotericin B has been found. There is some variation between mouse strains in their responsiveness to Am B. Work under this contract will be completed during this funding year.

Significance for Cancer Research (NCP Objective 6 Approach 1,4)
The possibility of using simple chemicals for the stimulation of the immune response must be thoroughly investigated.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $60,000

CONTRACT RESEARCH SUMMARY

Title: Multi-Test Device with Standardized Antigens to Assay Delayed Hypersensitivity

Principal Investigator: Gary L. Hein
Name/Address President
Performing Lincoln Laboratories, Inc.
Organization: Decatur, Illinois 62525

Contract Number: NO1-CB-43954
Starting Date: 06/30/74 Expiration Date: 07/29/74

Goal: To develop a multi-test device to be used in studying skin test responsiveness in patients receiving immunotherapy.

Approach: Standardized viral, bacterial, and fungal antigens will be developed in concentrated form which will permit their use on a multi-puncture skin test device. The skin testing device will be constructed so that multiple antigens may be applied simultaneously in a highly reproducible manner.

Progress: Three antigens have been developed in animal models and are now undergoing human testing. Food and Drug Administration guidelines are being fulfilled. The multitest device has been developed and is in production. After antigen concentrations are confirmed in normal volunteers, the skin test materials will be evaluated in cancer patients.

Significance for Cancer Research (NCP Objective 6 Approach 1)
The need for standardization of *in vivo* testing of patients is a fundamental problem in clinical immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: 6/13/74 - 7/9-10/74
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $125,245

CONTRACT RESEARCH SUMMARY

Title: Phase I Study of Effects of Immune Stimulants on Human Immune Responses

Principal Investigator: Charles G. Moertel, M.D.
Name/Address Gastrointestinal Oncology
Performing Mayo Clinic
Organization: Rochester, MN 55901

Contract Number: NO1-CB-53874
Starting Date: 06/01/75 Expiration Date: 5/31/76

Goal: Determine acute and chronic toxicity, maximum tolerated dose, effects on immune status, optimum immunostimulating dose, etc. for various immunotherapeutic agents.

Approach: Appropriately designed clinical trials will be utilized.

Progress: Phase I studies of lyophilized and fresh frozen BCG were well under way at the time of the first progress report.

Significance for Cancer Research (NCP Objective 6 Approach 4)
Phase I studies of immunotherapeutic agents are necessary for the proper study of the efficacy of such agents.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: Est. $145,000

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Human Cancer

Principal Investigator: Jordan U. Gutterman, M.D.
Name/Address Performing Organization: M. D. Anderson Hospital & Tumor Inst.
Univ. of Texas System Cancer Center
Houston, Texas 77025

Contract Number: N01-CB-33888
Starting Date: 10/25/75 Expiration Date: 10/24/76

Goal: To evaluate BCG as a form of immunotherapy in several types of local and disseminated human cancer.

Approach: Patients with wide-spread involvement of a given cancer in relatively good condition but with a uniformly poor prognosis will be randomized with due regard for age, sex, general condition, and other variables determined by the particular tumor. Statistical considerations to insure evaluable results will enter into the randomization and the determination of patient numbers to be entered into protocols.

Progress: Immunotherapy is being evaluated in melanoma, breast cancer, head and neck cancer and leukemia. Disease free interval, remission rates, remission duration and survival times are being measured. Immunologic measurements are made on many protocol patients.

Significance for Cancer Research (NCP Objective 6 Approach 1,4)
Because of the wide-spread casual application of immunotherapy to human patients, it is important to establish well designed eithical clinical protocols.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $409,000

CONTRACT RESEARCH SUMMARY

Title: Animal Models for Treatment of Minimal Residual Systemic Tumor

Principal Investigator: Gerald L. Bartlett, M.D., Ph.D.
Name/Address M. S. Hershey Medical Center
Performing Pennsylvania State University
Organization: Hershey, Pennsylvania 17033

Contract Number: N01-CB-33891
Starting Date: 11/01/75 Expiration Date: 10/31/76

Goal: To develop animal models for the assessment of the usefulness and parameters of administration of various types of immune stimulants as therapy for cancer.

Approach: Animals models with a direct applicability to human cancer will be studied for the effectiveness of various immunostimulants on their cancers. The immunotherapy will be given after tumor load has been reduced by surgery, radiation, or chemotherapy, and the tumor load will be quantifiable. Experimental variables will include strain of orgainism, dose, schedule, and route of administration.

Progress: Three tumor models are being evaluated. Systemic administration of BCG was not effective treatment for mice with the B16 melanoma or mice with the LSTRA lymphoma. Intralesional injection of Bordatella pertussis into mammary carcinoma retarded tumor growth. Future efforts under this contract will focus on maximizing observed immunotherapeutic effects.

Significance for Cancer Research (NCP Objective 6 Approach 1,4)
Evaluation of parameters of promising forms of immunotherapy in animals is necessary for optimum utilization in humans.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: 5/30/74
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $138,032

CONTRACT RESEARCH SUMMARY

Title: Graft Versus Tumor: A Model for Treatment of Carcinoma, Leukemia and Lymphoma

Principal Investigator: Mortimer M. Bortin, M.D.
Name/Address Winter Research Laboratory
Performing Mount Sinai Medical Center
Organization: Milwaukee, Wisconsin 53233

Contract Number: NO1-CB-33853
Starting Date: 08/25/75 Expiration Date: 11/24/75

Goal: To evaluate adoptive immunotherapy using histo-incompatible immunocompetent cells in the treatment of spontaneous leukemia-lymphoma in AKR mice.

Approach: Using spontaneous tumors in AKR mice: 1) combinations of chemotherapy and chemoradiotherapy will be evaluated for treatment of leukemia and for inducing immunosuppression; 2) H-2 incompatible immunocompetent cells will be administered to the immunosuppressed animals and graft versus host reaction will be monitored; 3) chemotherapy and chemoradiotherapy plus antiserum against the H-2 incompatible cells plust immune H-2 compatible bone marrow will be administered for purposes of rescue from the graft versus host reaction.

Progress: The investigator has established a colony of approximately 1500 AKR mice bearing spontaneous leukemia-lymphoma (SLL) for: treatment with one of 32 regimens employing chemotherapy or chemoradiotherapy; chemotherapy or chemoradiotherapy + transplant of foreign bone marrow cells from healthy mice; and untreated controls. Important findings include the observation that, while single high doses of chemoradiotherapy are associated with high mortality rates, a low initial dose of chemoradiotherapy followed by large doses of chemoradiotherapy are tolerated well, and occasionally, has resulted in marked tumor load reduction. Experiments are underway to administer H-2 incompatible immunocompetent cells to immunosuppressed leukemia-lymphoma mice with subsequent monitoring of graft versus host reactions and their effects on tumor growth. Early results have been encouraging in 2 groups. Evaluation of chemotherapy or chemoradiotherapy and antiserum against H-2 incompatible cells + administration of immune H-2 compatible bone marrow for purposes of rescue from the graft versus host reaction is being undertaken.

Significance for Cancer Research (NCP Objective 6 Approach 1)
Development of an animal model with immunocompetent cells with "rescue" from graft versus host disease could be highly effective in human immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $16,900

CONTRACT RESEARCH SUMMARY

Title: Chemoimmunotherapy of Acute Myelocytic Leukemia

Principal Investigators: James F. Holland, M.D. and
Name/Address J. George Bekesi, Ph.D.
Performing Mt. Sinai School of Medicine
Organization: 5th Avenue and 100th Street
New York, New York 10029

Contract Number: N01-CB-43879
Starting Date: 06/30/74 Expiration Date: 12/20/76

Goal: Evaluate Neuraminidase-treated allogeneic cells and MER in human acute myelocytic leukemia (AML).

Approach: Neuraminidase (VCN) treated allogeneic cells and MER will be evaluated in an appropriately designed, randomized study of AML patients after remission induction with cytosine arabinoside and Daunorubicin. Patients will be studied with both in vivo and in vitro immunologic tests.

Progress: Patients are being entered into a defined clinical protocol. Preliminary analysis will be made in the next contracting period.

Significance for Cancer Research (NCP Objective 6 Approach 1,4)
Suggestive early leads in immunotherapy of AML require that vigorous application of other possible modalities to this disease be undertaken.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: 4/5/74
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $293,971

CONTRACT RESEARCH SUMMARY

Title: Qualitative Assays of Monocyte-Macrophage Function

Principal Investigator: Gerald W. King, M.D.
Name/Address Performing Organization: Division of Hematology & Oncology
Ohio State University Hospitals
410 West 10th Avenue
Columbus, Ohio 43210

Contract Number: NO1-CB-53936
Starting Date: 6/16/75 Expiration Date: 6/15/76

Goal: Apply monocyte-macrophage studies to the evaluation of cancer patients.

Approach: Old and new methodology is being modified for the purpose of studying cancer patients, especially those receiving immunotherapy.

Progress: Three established assays of monocyte function have been evaluated in 20 untreated cancer patients and 17 successfully treated lymphoma patients. Early results suggests that untreated cancer patients may have a significant impairment in monocyte candidicidal activity. Three new assays are being developed and are nearly ready for application to the study of patients.

Significance for Cancer Research (NCP Objective 2,4,6 Approach 1,2,4)
Evaluation of macrophage activity is necessary for the study of immonotherapeutic agents.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: Est. $82,000

CONTRACT RESEARCH SUMMARY

Title: New Approaches to Immunotherapy

Principal Investigator: Jerald J. Killion, Ph.D.
Name/Address Performing Organization: Oklahoma Medical Research Foundation
825 North East 13th Street
Oklahoma City, Oklahoma 73104

Contract Number: NO1-CB-53937
Starting Date: 6/27/75 Expiration Date: 6/26/76

Goal: To study the usefulness of antigenic subpopulation of tumor cells in immunotherapy.

Approach: Tumor cells will be subcategorized on the basis of characteristics of cell surface, carbohydrates, and of drug-resistance. Subpopulations will be used in therapeutic protocols in animal tumor systems.

Progress: The investigators have early evidence that antigenic subpopulations of tumor cells may offer advantages in immunotherapy. Future work will be directed towards establishing reproducibility and generalizability of the early results.

Significance for Cancer Research (NCP Objective 2,4,6 Approach 1,2,4)
Specific Immunotherapy with tumors may offer substantial advantages over non-specific immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: Est. $70,000

CONTRACT RESEARCH SUMMARY

Title: Transfer Factor Therapy of Sarcoma

Principal Investigator:
Name/Address
Performing
Organization:

Albert F. LoBuglio, M.D.
Ohio State University Research Fdn.
410 West 10th Avenue
Columbus, Ohio 43210

Contract Number: NO1-CB-43878
Starting Date: 04/01/74 Expiration Date: 3/31/75

Goal: Evaluate transfer factor as a therapeutic agent in human sarcomas.

Approach: Transfer factor will be derived by a chromatographic isolation technique from the leukocytes of relatives of tumor patients who have been shown to have tumor immunity with in vitro assays. Several different types of sarcomas will be studied in appropriately designed protocols.

Progress: The therapy strategy is to administer large amounts of transfer factor in a short time to patients in the postoperative period (1-3 months post-op) in order to accentuate tumor immunity when tumor cell numbers should be at a minimum. Clinical and laboratory evaluation on specific tumor immunity and general immune evaluation is proceeding satisfactorily. Present data suggest that column-purified TF does not transfer "markers" skin test reactivity and that the MIF assay is less useful than the cytotoxicity assay. Because of low patient accrual, this contract will end after completion of certain aspects of the ongoing work.

Significance for Cancer Research (NCP Objective 2,4 Approach 1,2,3,4)
Transfer factor may be a relatively nontoxic and possibly highly specific approach to immunotherapy of cancer.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $90,000 (Terminal year)

CONTRACT RESEARCH SUMMARY

Title: Animal Models for Evaluation of Therapy with Neuraminidase Treated Cells

Principal Investigator: G. Mark Kollmorgen, Ph.D.
Name/Address Performing Organization: Oklahoma Medical Research Foundation
825 Northeast 13th Street
Oklahoma City, Oklahoma 73104

Contract Number: NO1-CB-43864
Starting Date: 11/01/75 Expiration Date: 10/31/76

Goal: To explore the parameters of therapy with neuraminidase treated autologous or isogeneic tumor cells.

Approach: Using known successful animal models, parameters of immunotherapy with tumor cells such as cell number, route and schedule of administration, methods and duration of cell storage, and use of tissue culture cells will be studied. In addition, potential new model systems for evaluation of this form of immunotherapy will be explored. Such systems will be required to have high applicability to human cancer; that is, therapy will be evaluated in the presence of established tumors and the tumors will be of relatively recent origin.

Progress: The contractor has generated considerable information relating to the combination of chemotherapeutic agents and immunotherapeutic agents in one tumor model. The work will be completed during this contracting period.

Significance for Cancer Research (NCP Objective 6 Approach 1,4)
Animal models for immonotherapy will not only contribute to the understanding of immunotherapy but also to the problems of diagnosis of the quantity of malignant disease and the evaluation of normal body mechanisms of defense against malignant disease.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: 5/21/73
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $132,841 (Terminal year)

CONTRACT RESEARCH SUMMARY

Title: Role of Macrophages in the Immune Response to Tumors

Principal Investigator: James L. Krahenbuhl, Ph.D.
Name/Address Palo Alto Medical Research Fdn.
Performing 860 Bryant Street
Organization: Palo Alto, California 94301

Contract Number: N01-CB-43873
Starting Date: 05/01/76 Expiration Date: 04/30/77

Goal: To study the role of macrophages in the immune response to tumors, particularly in relation to immunotherapy, both in animals and in man.

Approach: Guinea pig and mouse peritoneal macrophages and human peripheral blood monocytes and monocyte derived macrophages will be studied for their capacity to adversely affect a number of syngeneic and allogeneic tumor target cells. Kinetics of the response of activated macrophages will be examined and the macrophage activation related to acute and chronic stages of infection will be evaluated. Cell mediated immune responses of animals and patients to Toxoplasma antigens, streptokinase-streptodornase, and PPD will be evaluated by skin tests and by in vitro techniques.

Progress: Cytostatic effects of peritoneal macrophages and peritoneal lymphocytes are being compared in animals infected with intracellular protozoans. Macrophages were found to be nonspecifically cytostatic for a variety of tumor cells. Infection with Listeria or treatment with C. parvan was found to induce a transient state of macrophage activation when compared with infection with Toxoplasma. The cytostasis appears to result from direct contact between the activated macrophage and target cells. Several methods designed to evaluate viability of target cells have shown that activated macrophages appear to be highly cytostatic but not cytotoxic. Some of these methodologies will be applied to the study of human monocytes in the next contracting period.

Significance for Cancer Research (NCP Objective 2,4,6 Approach 1,2,4)
Understanding mechanisms of macrophage activity has high relevance to the timing of immunotherapeutic manipulations.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: Est. $110,000 (Terminal year)

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Accessible Neoplasms and Analysis of Immune Status In vivo

Principal Investigator: Edmund Klein, M.D.
Name/Address Performing Organization: Research Foundation of the State University of New York
Buffalo, New York 14203

Contract Number: NO1-CP-33373
Starting Date: 03/21/75 Expiration Date: 02/20/77

Goal: Exploratory studies in the utilization of topical immunotherapy and correlation with in vivo measurement of immune status.

Approach: Patients with various neoplasms originating or metastasizing to the skin will be entered into specific clinical protocols for application of topical immunotherapeutic agents. Some patients will receive BCG by scarification. Multiple antigens will be used for skin testing on all patients and the skin reactivity will be correlated with clinical status and immunotherapy administration.

Progress: The contractor has developed clinical protocols and entered patients into these protocols as follows: 1) Protocol #438-BCG Effects on Skin Test Responses and Incidence of Recurrence in Surgical Remission of Malignant Melanoma. Forty-seven patients are evaluable in this study and it will be completed in one year. 2) Protocol #69-273-Double Blind Comparison of Topical 5-FU, PPD, and Combination of 5-FU/PPD on Cutaneous Neoplasms. No differences was found between the therapeutic agents and this protocol is now terminated.

Significance for Cancer Research (NCP Objective 2,5,6 Approach 1,3,4)
Detailed information regarding results of skin testing in relation to clinical status and therapy provide vital feedback for therapeutic manipulations.

Project Officer: Dorothy Windhorst, M.D. and Charles Boone, M.D.
Program: Immunotherapy Site Visit Date: 10/31/74
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $50,000 (Terminating)

CONTRACT RESEARCH SUMMARY

Title: Macrophage Activation in Tumor Immunity and Immunotherapy of Rat Mammary Tumor

Principal Investigator: W. Hallowell Churchill, Jr., M.D.
Name/Address Robert B. Brigham Hospital
Performing 125 Parker Hill Avenue
Organization: Boston, Massachusetts 02120

Contract Number: NO1-CB-33896
Starting Date: 11/01/75 Expiration Date: 10/31/76

Goal: To study the mechanism of tumor immunogenicity in order to design a more effective and rational program of tumor immunotherapy in man.

Approach: The contractor will evaluate the ability of macrophages to kill tumor cells after in vitro activation by lymphocyte mediators, and determine whether macrophages activated by adjuvants in vivo show differences in tumor cell killing activity in vitro. In addition, he will extend these studies to human monocytes.

Progress: The investigators have established new systems for the study of macrophage cytotoxicity for tumor cells that will facilitate dissection of the mechanisms of tumor cytotoxicity and how such mechanisms might be stimulated for immunotherapeutic purposes. Suspension cultures of peritoneal exudate cells activated by lymphocyte mediators appear to kill embryonic fibroblasts and tumor cells to the same extent.

Significance for Cancer Research (NCP Objective 6 Approach 1,4)
Immunotherapeutic manipulations in animals are needed to evaluate multiple parameters of immunotherapy that cannot satisfactorily be tested in humans.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $109,300 (Terminating)

CONTRACT RESEARCH SUMMARY

Title: Quantitative Assays of Monocyte-Macrophage Function

Principal Investigator: John R. David, M.D.
Name/Address Department of Medicine
Performing Robert B. Brigham Hospital
Organization: 125 Parker Hill Avenue
Boston, MA 02120

Contract Number: NO1-CB-53869
Starting Date: 6/01/75 Expiration Date: 5/31/76

Goal: To develop new assays for human monocyte-macrophage function.

Approach: Guinea pig systems shall be used for exploratory work and promising assays will be applied to human materials with emphasis on miniaturization.

Progress: A method has been devised to obtain highly enriched human monocyte populations. Monocyte receptors for Fc and complement are being quantified on human monocytes, as are receptors for lymphocytes. Methods for studying the transport of small molecules following monocyte activation have been developed. Guinea pig macrophage activation is being evaluated by the study of pinocytosis and ecto-enzyme changes.

Significance for Cancer Research (NCP Objective 2,4,6 Approach 1,2,4)
Evaluation of macrophage activity may determine the effectiveness of immunotherapeutic manipulations.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: Est. $107,000

CONTRACT RESEARCH SUMMARY

Title: Effect of Transfer Factor on Virus-induced Tumors in Marmoset Monkeys

Principal Investigator:
Name/Address
Performing
Organization:

Peter Baram, Ph.D.
Div. of Microbiology & Immunology
Rush-Presbyterian-St. Luke's Medical Center
Chicago, Illinois 60612

Contract Number: NO1-CB-43951
Starting Date: 06/28/75 Expiration Date: 06/27/76

Goal: Utilize a subhuman primate system to evaluate a promising mode of immunotherapy.

Approach: Two different virus-induced tumors of marmoset monkeys will be utilized to evaluate therapeutic effect of transfer factor, its specificity, and the optimum dose and schedule of administration. Transfer factor will be compared with immune RNA in one of these studies.

Progress: The investigator has early evidence suggesting that he has achieved lymphocyte transformation with marmoset lymphocytes; acquired reproducibility in the marmoset MIF assays; and achieved transfer of delayed hypersensitivity to KLH as measured by the MIF assay in two of four rhesus monkeys using KLH reactive dialyzable transfer factor (TF_d).

Significance for Cancer Research (NCP Objective 2,4 Approach 1,2,3,4)
Subcellular fractions such as transfer factor and immune RNA may be relatively nontoxic and possibly highly specific approaches to cancer immunotherapy. Lack of suitable models in smaller animals requires that primate systems be studied.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: 4/29/74
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: Est. $110,000

CONTRACT RESEARCH SUMMARY

Title: Role of Circulating Tumor Antigens (Immunotherapy)

Principal Investigator: Joseph D. Feldman, M.D.
Name/Address Performing Organization: Scripps Clinic and Research Foundation
476 Prospect Street
La Jolla, California 92037

Contract Number: NO1-CB-43874
Starting Date: 03/01/76 Expiration Date: 02/28/77

Goal: To evaluate the effects that circulating tumor antigens may have on immunotherapeutic maneuvers.

Approach: Several different antigens associated with a viral tumor system will be isolated. The metabolism of these antigens and the circulatin in hosts with regressing and progressing tumors will be measured. The immune response to each category of antigen will be analyzed and the antigen will be utilized to evaluate their capacities to accentuate destruction of the tumor.

Progress: Tumor antigens, viral antigens and histocompatibility antigens in a rat model system are being evaluated in relation to the disease status of the tumor bearing animals.

Significance for Cancer Research (NCP Objective 2,6 Approach 1,5,3,4)
Knowledge of how circulating antigens may influence immunotherapy is important for the design of courses of immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $103,424

CONTRACT RESEARCH SUMMARY

Title: Effects of Immune Stimulants on Human Immune Response

Principal Investigator: Yashar Hirshaut, M.D.
Name/Address Performing Organization: Sloan-Kettering Institute for Cancer Research
425 East 67th Street
New York, New York 10021

Contract Number: NO1-CB-53970
Starting Date: 05/16/75 Expiration Date: 05/15/76

Goal: To provide a resource for the evaluation of selected immunotherapeutic agents for acute and chronic toxicity, maximum tolerated dose, and immunological effects.

Approach: Experimental plans for specific Phase I studies of specific immunotherapeutic agents are developed on the background of a master protocol involving the evaluation of maximum acute and chronic dosages of designated immunotherapeutic agents.

Progress: Phase I evaluation of C. parvum by the intravenous subcutaneous, and intralesional route will be completed early in the second contract year. Similarly, the study of Levamisole will also be completed. Once the evaluation of these agents is completed the contractor will initiate studies with new immunotherapeutic agents after consultation with the project officer.

Significance for Cancer Research (NCP Objective 2,4 Approach 1,2,3,4)
Phase I studies are needed in order to develop well designed Phase II clinical trials.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: Est. $140,000

CONTRACT RESEARCH SUMMARY

Title: Evaluation of C. Parvum in Disseminated Carcinoma of the Breast

Principal Investigator: Carl Pinsky, M.D.
Name/Address Performing Organization: Sloan-Kettering Institute Memorial Hospital
New York, New York 10021

Contract Number: NO1-CB-53873
Starting Date: 06/30/75 Expiration Date: 6/29/76

Goal: Evaluate the usefulness of C. parvum in breast cancer.

Approach: C. parvum will be administered in the context of defined clinical protocols.

Progress: Patient accrual rate in this contract should permit the analysis of several parameters of the administration of C. parvum. At present follow-up is too short to permit definitive statements.

Significance for Cancer Research (NCP Objective 6 Approach 4)
The usefulness of C. parvum needs to be defined in a randomized trial.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: Est. $110,000

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy for Tumor Patients with No Detectable Disease or Minimal Tumor Burden

Principal Investigator: Harold J. Wanebo, M.D.
Name/Address Performing Organization: Sloan-Kettering Institute for Cancer Research
410 East 68th Street
New York, New York 10021

Contract Number: NO1-CB-53875
Starting Date: 06/30/75 Expiration Date: 06/29/76

Goal: Evaluate immonotherapy in patients with head and neck cancer.

Approach: Levamisole will be administered in the context of a defined clinical protocol to evaluate its usefulness in the therapy of patients with head and neck cancer.

Progress: Patient accrual rate into the clinical protocol is proceeding well. The short time interval does not permit any analysis of the value of levamisole or the usefulness of in vitro assays for following this group of patients.

Significance for Cancer Research (NCP Objective 6 Approach 4)
Head and neck cancer patients appear to be immunodepressed. Levamisole may be useful in improving this state.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: Est. $80,000

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy with In Vitro Lymphocyte Sensitization

Principal Investigator: Henry S. Kaplan, M.D.
Name/Address Performing Organization: Department of Radiology
Stanford University School of Medicine
Stanford, California 94305

Contract Number: NO1-CB-63988
Starting Date: 9/01/75 Expiration Date: 8/31/76

Goal: To evaluate the usefulness of lymphocytes snesitized in vitro for the immunotherapy of cancer.

Approach: The immunotherapeutic efficacy of sensitized lymphocytes will be measured against autochthonous murine lymphomas: optimal conditions for collecting, storing, and sensitizing fresh human peripheral blood lymphocytes against human tumors will be developed; and, ultimately information gained in mouse model systems will be applied to immunotherapy of human cancers.

Progress: New Contract

Significance for Cancer Research (NCP Objective 2,4 Approach 1,2,3,4)
Evaluation of methodologies for specific sensitization against tumors is needed for immunotherapeutic efforts.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: Est. $75,000

CONTRACT RESEARCH SUMMARY

Title: Study Mycobacteria as Inhibitors of Tumor Cell Growth

Principal Investigator: George B. Mackaness, Ph.D., M.D.
Name/Address Trudeau Institute Inc.
Performing Immunobiological Research Laboratories
Organization: Saranac Lake, New York 12983

Contract Number: NO1-CB-23221
Starting Date: 02/01/76 Expiration Date: 01/31/77

Goal: Evaluate various highly characterized strains of BCG for their capacity to suppress tumor growth in guinea pigs and mice; compare C. parvum with BCG in the model systems.

Approach: The biological properties of different strains of BCG grown under different circumstances and preserved in various ways will be examined in terms of immunopotentiating and tumor-suppressive activities.

Progress: The immunotherapeutic and immunopotentiating properties of five strains of fresh-frozen (FF) and freeze-dried (FD) BCG vaccines are being compared by three different assay procedures. C. parvum has been compared with these strains of BCG in one tumor systems and for its capacity to affect various parameters of the immune response administered intravenously and intraperitoneally.

Significance for Cancer Research (NCP Objective 6 Approach 1,4)
Preclinical evaluation of mechanisms of action and comparative efficacies of promising immunotherapeutic agents is necessary for efficient design of clinical trials.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $185,001

CONTRACT RESEARCH SUMMARY

Title: The Production of an Improved BCG Vaccine

Principal Investigator: George B. Mackaness, M.D., Ph.D.
Name/Address Tradeau Institute, Inc.
Performing Immunobiological Research Laboratories
Organization: Saranac Lake, New York 12983

Contract Number: NO1-CB-53885
Starting Date: 08/23/75 Expiration Date: 08/22/76

Goal: Develop production methods for an improved BCG which will meet FDA standards for human use.

Approach: BCG is grown in suspension culture in the absence of any animal proteins or other significantly antigenic materials. It is harvested at a defined stage of growth and frozen for distribution for clinical studies.

Progress: Batches of the fresh frozen BCG suitable for human use under an FDA Investigational New Drug permit are being distributed to clinical investigators.

Significance for Cancer Research (NCP Objective 6 Approach 1)
The superiority of a specific preparation of this agent for immunotherapy has been suggested by preclinical experiments and human trials are awaiting large scale production of this material.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $54,952

CONTRACT RESEARCH SUMMARY

Title: Specific and non-specific Immunotherapy in Skeletal and Soft Tissue Sarcomas

Principal Investigator: Frederick Eilber, M.D.
Name/Address Division of Oncology
Performing Room 54-140 Shirley
Organization: UCLA School of Medicine
Center for the Health Sciences
Los Angeles, CA 90024

Contract Number: NO1-CB-53941
Starting Date: 6/16/75 Expiration Date: 6/15/76

Goal: Evaluate immunotherapy in skeletal or soft tissue sarcomas.

Approach: Patients will be entered into well-defined clinical protocols.

Progress: New Contract. Early reports indicate that patient accrual is proceeding well under this contract.

Significance for Cancer Research (NCP Objective 6 Approach 4)
Immunologic studies suggest that sarcomas may offer a particularly useful human tumor for evaluation of immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: Est. $110,000

CONTRACT RESEARCH SUMMARY

Title: Human Melanoma: Evaluation of BCG Immunotherapy

Principal Investigator: Donald L. Morton, M.D.
Name/Address Department of Surgery
Performing University of California-Los Angeles
Organization: Los Angeles, California 90024

Contract Number: NO1-CB-43852
Starting Date: 08/08/74 Expiration Date: 08/07/75

Goal: Conduct a suitably randomized study to evaluate promising preliminary evidence that BCG therapy is useful in melanoma.

Approach: Patients with a diagnosis of melanoma who have proven involvement of regional lymph nodes and whose disease has been totally removed as far as can be determined are appropriately randomized into treatment groups with due regard for sex, age, and disease classification. The three treatment arms are: 1) no further therapy; 2) BCG by time injection; and 3) BCG and melanoma cells intradermally. Clinical studies necessary to establish the diagnosis and document the patient's general health status, tumor status, toxicity of therapy, time to relapse, and survival time are being made. Some patients' immunologic status is being followed with a variety of *in vitro* assays.

Progress: The number of patients being entered into the protocol is outstanding. Presently, an average of eight patients per month are being entered, leading to projections that there will be a total of 180-200 patients by the end of the second contract period. Preliminary evaluation of the patient status has not given conclusive answers.

Significance for Cancer Research (NCP Objective 6 Approach 4)
This is a direct extension of important preliminary observations regarding immunotherapy in human melanoma. This contract will permit a suitable controlled, randomized clinical trial which should resolve a number of questions about this type of immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: 3/10/74
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: Est. $255,000

CONTRACT RESEARCH SUMMARY

Title: Plasma Therapy of Osteosarcoma

Principal Investigator: Richard T. Smigh, M.D.
Name/Address Department of Pathology
Performing College of Medicine
Organization: University of Florida
Gainesville, FL 32610

Contract Number: NO1-CB-53933
Starting Date: 6/16/75 Expiration Date: 6/15/76

Goal: Evaluate the therapeutic efficacy of plasma transfusions in osteosarcoma.

Approach: Plasma having in vitro anti-osteosarcoma activity will be administered to osteosarcoma patients after primary surgery in the context of clinical protocols.

Progress: Two promising assays for apparent tumor specific immunity against autologous tumors have been developed. The rate of patient accrual to the clinical protocol may be insufficient to answer the immunotherapeutic questions.

Significance for Cancer Research (NCP Objective 6 Approach 4)
Antibody transfer from presumed immune individuals to patients bearing cancers is a promising mode of immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $55,000

CONTRACT RESEARCH SUMMARY

Title: Human Lung Cancer - Evaluation of BCG Therapy

Principal Investigator: Robert H. Waldman, M.D.
Name/Address Performing Organization: University of Florida College of Medicine, Gainesville, Florida 32601

Contract Number: NO1-CB-43857
Starting Date: 11/15/75 Expiration Date: 11/14/76

Goal: Evaluate immunotherapy in cancer patients with a poor prognosis but in otherwise good health.

Approach: Newly diagnosed and previously untreated patients with oat cell and squamous cell carcinomas of the lung will be randomized with due regard for age, sex, and known variables for survival in the specific tumor. BCG will be administered by a schedule to be arranged with the Project Officer. All clinical studies necessary to establish the diagnosis and the extent of the cancer, as well as periodic documentation of the patients' level of health, tumor status, reaction to therapy, deviations from protocol, time to relapse, and survival time will be made. Certain specified immunologic tests will also be conducted.

Progress: During the initial contract period, the contractor administered 86 aerosolized BCG doses to 20 patients with proven non-resectable lung cancer in a Phase I study. A substantial degree of pulmonary toxicity was noted in this study, and a protocol for mitigating this toxicity was developed and tested. Because of decreased patient accrual to the study, this contract will terminate in FY77.

Significance for Cancer Research (NCP Objective 6 Approach 1,4)
Because of the possibility that immunotherapy offers a distinct additive to other kinds of therapy, there is an urgent need to evaluate this therapy in patients who are not yet compromised by the extent of their disease.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: 5/18/74
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $50,000

CONTRACT RESEARCH SUMMARY

Title: Alpha-Fetoprotein in Immunotherapy of Experimental Hepatomas

Principal Investigator: Erkki Ruoslahti, M.D.
Name/Address Performing Organization: Dept. of Serology and Bacteriology
University of Helsinki
SF-00290 Helsinki 29
Finland

Contract Number: NO1-CB-53872
Starting Date: 6/16/75 Expiration Date: 6/15/76

Goal: To evaluate the usefulness of antibodies against tumor antigens in an experimental animal model.

Approach: Antibodies against alpha-fetoprotein will be produced and evaluated as therapeutic agents in animals bearing experimental tumors.

Progress: New Contract. A final progress report will be filed at the end of the contracting period.

Significance for Cancer Research (NCP Objective 6 Approach 4)
Humoral immunity may offer significant advantages in the treatment of cancer.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: Est. 0 (Contract will terminate after one year.)

CONTRACT RESEARCH SUMMARY

Title: Anti-tumor Activity of RNA Extract and Transfer Factor

Principal Investigator: Sheldon Dray, M.D., Ph.D.
Name/Address Professor & Head, Dept. of Microbiology
Performing University of Illinois School of Basic
Organization: Medical Sciences
Chicago, Illinois 60680

Contract Number: NO1-CP-23205
Starting Date: 8/01/74 Expiration Date: 8/31/75

Goal: Evaluate immune RNA extracts of lymphocytes for capacity to exert an immunotherapeutic action.

Approach: RNA rich extracts from lymphoid tissues of strain 2 guinea pigs immune to Line 10 hepatoma and from xenogeneic sources are evaluated in vitro for therapeutic efficacy.

Progress: The investigators report that testablished five-day old tumors will regress when treated intralesionally with a mixture of normal peritoneal exudate cells, anti-tumor immune RNA, and 1 ml. soluble tumor-specific antigen. The investigators also have early evidence that tumor nodules remote from the injection site may also regress. This contract expired August 31, 1975.

Significance for Cancer Research)NCP Objective 2,4 Approach 1,3)
Immune RNA may be a relatively nontoxic and possibly highly specific approach to immunotherapy of human cancer.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy: Role of Circulating Tumor Antigens

Principal Investigator: Charles F. McKhann, M.D.
Name/Address Department of Surgery
Performing University of Minnesota Medical School
Organization: Minneapolis, Minnesota 55455

Contract Number: NO1-CB-43946
Starting Date: 05/01/75 Expiration Date: 04/30/76

Goal: To study the effects of circulating tumor antigens in relation to tumor size and response to immunotherapy.

Approach: Antigen and antibody complexes in the circulation of normal and tumor-bearing mice will be evaluated in relation to tumor size and in vitro measurements of immune responsiveness. Effects of irradiation in this system will also be studied. Tumor antigen, antibody to tumor antigen, and antigen-antibody complexes will be studied for stimulation and blocking of stimulation of lymphocyte dependent antibody and cellular cytotoxicity.

Progress: Utilizing a mouse system with 2 methylcholanthrene induced tumors the investigator has shown that lymph node cells from tumor-bearing mice will react in vitro to the specific tumor if the tumor load is small. However, lymph node cells from mice with tumors greater than 1 cm. in diameter will not react. Longitudinal studies suggest that systemic immunity is present and vigorous until approximately 15 days after inoculation of tumors in experimental animals. The subsequent dramatic suppression of cellular immunity appears to coincide with the time when tumor antigens can be detected in the circulation. Methodology is being developed for tumor antigne purification.

Significance for Cancer Research (NCP Objective 2,6 Approach 1,5,3,4)
Kinetics of circulating tumor antigens may have a profound effect on proper design of courses of immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: Est. $90,000 (Terminal year)

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy through Inhibition of Antibody Production

Principal Investigator: Charles F. McKhann, M.D.
Name/Address Department of Surgery
Performing University of Minnesota
Organization: Medical School
Minneapolis, Minnesota 55455

Contract Number: NO1-CB-92061
Starting Date: 06/14/75 Expiration Date: 06/13/76

Goal: Evaluate the role of immunity in the success or failure of tumors and determine how best to manipulate the immune response to the genefit of the host.

Approach: Manipulate the immune response of tumor-bearing animals by developing antibodies against plasma cells and B cells. Evaluate any therapeutic effects of this manipulation in tumor-bearing animals.

Progress: Antibody directed specifically against plasma cells was tested extensively for inhibition of tumor growth. Anti-plasma cell serum (APS) worked well in some tumor systems but not in others. Even where it was effective, a modest increase in the size of the tumor challenge dose overcame the APS inhibition. In contrast, the same cytotoxic anti-sera have been useful when used for in vitro maniuplations of anti-tumor immune responses. This work will be concluded in the present contracting period.

Significance for Cancer Research (NCP Objective 2,6 Approach 1,5,3,4)
Knowledge of how circulating antigens may influence immunotherapy is important for the design of courses of immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: 0 (Terminating)

CONTRACT RESEARCH SUMMARY

Title: Role of Macrophages in Tumor Immunology

Principal Investigator: Jon R. Schmidtke, Ph.D.
Name/Address Department of Surgery
Performing University of Minnesota Medical School
Organization: Minneapolis, Minnesota 55455

Contract Number: N01-CB-43948
Starting Date: 06/01/75 Expiration Date: 05/31/76

Goal: Develop assays and models for evaluation of macrophages in immunotherapy.

Approach: Syngeneic tumors in mice will be utilized to study the role of macrophage function in both the afferent and efferent phases of the immune response. Studies will include evaluation of phagocytosis, catabolism of tumor antigens, the capacity of macrophages to transfer immunogenicity, tumoricidal capacity of macrophages and study of MIF effects. Human macrophages will be evaluated with a number of in vitro assays.

Progress: Animal studies have suggested the possibility of utilizing macrophages ormacrophage associated materials as therapeutic agents, and further work on this question is underway. Methodologies for evaluation of human monocyte function are developing appropriately.

Significance for Cancer Research (NCP Objective 2,4,6 Approach 1,2,4)
Evaluation of macrophage activity is important to the timing of immunotherapeutic manipulations.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: Est. $72,000

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Cancer in Man

Principal Investigator: Richard L. Simmons, M.D.
Name/Address Department of Surgery
Performing University of Minnesota Med. School
Organization Minneapolis, Minnesota 55455

Contract Number: N01-CB-23885
Starting Date: 02/01/76 Expiration Date: 01/31/77

Goal: To test the usefulness of immunization with autochthonous, neuraminidase treated cells in therapy of patients with widely disseminated cancer not amenable to other techniques of treatment.

Approach: This will be done by 1) determining the immunologic competence of the patient; 2) determining the immunologic reactivity of the patient to his own and similar tumors; 3) instituting the trial of therapy with neuraminidase treated tumor cells derived from the patient's own cancer; 4) re-determining the patient's immunologic competence at intervals during the course of treatment; 6) utilizing the criteria of objective remission used by cooperative chemotherapy groups in following the tumors in these patients; and 7) discontinuing therapy whenever lack of response is demonstrated. Patients will be limited to those with melanoma and sarcoma who have been demonstrated to have residual tumor that is not amenable to further treatment with either chemotherapy or surgery. In addition, these patients will have been demonstrated to have good immunologic responses.

Progress: There are currently 93 melanoma and 27 sarcoma patients being studied. The protocol results are not yet ready to be evaluated.

Significance for Cancer Research (NCP Objective 6 Approach 4)
Modified autochthonous tumor cells have been shown to be promising immunotherapeutic agents in animals. This contract attempts to apply the animal work to human cancer patients.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: 12/5-6/74
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: Est. $90,000

CONTRACT RESEARCH SUMMARY

Title: Hapten Treatment of Cancer

Principal Investigator: Kazuhiko Arai, M.D.
Name/Address The Graduate Hospital
Performing University of Pennsylvania School
Organization: of Medicine
Philadelphia, Pennsylvania 19146

Contract Number: NO1-CB-43967
Starting Date: 05/16/76 Expiration Date: 05/15/77

Goal: To evaluate L-phenylalanine plus antiserum in a syngeneic rat system.

Approach: Protocols utilizing rats which have previously indicated that intratumoral injection of L-phenylalanine mustard plus I.V. injection of antiserum against tumor modified with L-phenylalanine mustard has a therapeutic effect will be used to study syngeneic rat tumors in vivo.

Progress: The investigator has established two syngeneic tumors in two Lewis rat strains and has evaluated methods of tumor preparation for inoculation and the effects of preparation techniques on the antigenicity of the conjugates. Using both syngeneic and xenogeneic antisera, the investigator has demonstrated decreased growth in tumors previously injected with phenylalanine when the animal is given the antiserum IV.

Significance for Cancer Research (NCP Objective 2,6 Approach 1,4)
This work will evaluate a promising animal model in a system potentially applicable to human tumors.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: Est. $75,000 (Terminal year)

CONTRACT RESEARCH SUMMARY

Title: Neuraminidase Effectiveness in Tumor Immunotherapy and Mechanism of its Effect

Principal Investigator: Gabriel J. Gasic, M.D.
Name/Address Department of Pathology
Performing University of Pennsylvania School
Organization: of Medicine
Philadelphia, Pennsylvania 19104

Contract Number: NO1-CB-33860
Starting Date: 08/25/74 Expiration Date: 08/24/75

Goal: To evaluate neuraminidase treated tumor cells in immunotherapy by establishing several animal tumor models and then examining the variables of dose, route of injection, etc., in these models. To examine the capacity of neuraminidase treatment in therapy of postsurgical metastatic cancer.

Approach: 1) Test the effectiveness of neuraminidase-treated tumor cells as immunotherapy in two spontaneous and three methylcholanthrene induced mouse tumors. 2) Using model systems in which the neuraminidase treatment is effective, the following parameters will be evaluated: dose of treated tumor cells, numbers of successive subcutaneous injections, route of treatment, and time of administration after tumor grafting. 3) Evaluation of the effectiveness of neuraminidase-treated tumor cells in immunotherapy of metastatic tumor using metastatic tumor cells. 4) Examination of the effectiveness of neuraminidase modified cells in relation to the natural immunogenicity of certain target tumors.

Progress: Successful application of cholera neuraminidase (VCN) in cancer therapy has been limited to a few tumor systems. To investigate whether other tumors may also be susceptible to this therapy, chemical tumors were induced in Balb/c (12A9 urinary bladder carcinoma), B10D2 new (MC2 fibrosarcoma), C3H/He (MC43 fibrosarcoma), and in C57BL/6 (MC1 fibrosarcoma) mice. Two spontaneous tumors (Mammary #4 and #5) originated in C3H/He female mice. In 8 experiments, mice were treated with 6 injections s.c. every other day of 10^6 VCN- and mitomycin-treated tumor cells. In one experiment B10D2 new and B10D2 old mice received only 3 injections of the immunogen. Controls were injected with medium M199 or with tumor cells incubated with heat-inactivated VCN and mitomycin C. Results indicated that injection of VCN-treated tumor cells: 1) achieved no beneficial therapeutic effect in mammary tumors #4 and #5 and in 12A9 tumor; 2) induced no tumor regression but reduced tumor growth &/or prolonged survival. This was observed in MC43 fibrosarcoma and MC2 fibrosarcoma; and 3) produced some total tumor regression or contributed to tumor growth slowdown &/or prolonged survival in MC1 fibrosarcoma, a highly immunogenic tumor.

Significance for Cancer Research (NCP Objective 6 Approach 1)
Manipulation of autologous tumor with neuraminidase in animals has direct relevance to tumor immunotherapy in man.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $32,933 (Terminated)

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy: Mechanism of Action of Immunopotentiators

Principal Investigator: Alan M. Kaplan, Ph.D.
Name/Address Medical College of Virginia
Performing Virginia Commonwealth University
Organization: Richmond, Virginia 23298

Contract Number: NO1-CB-43877
Starting Date: 03/15/76 Expiration Date: 03/14/77

Goal: To evaluate the immunologic effects of immunopotentiators in relation to their immunotherapeutic capacity.

Approach: The immunologic action of selected immunopotentiators will be evaluated in model systems for antibody production and cellular immunity. The changes in these responses towards tumors from tumor bearing mice will be measured and compared with the model system. In addition, the effectiveness of the immunopotentiators in the treatment of tumors after using surgery or chemotherapy to remove the bulk of the tumor will be evaluated.

Progress: Pyran copolymer, polyacrylic acidmaleic anhydride, and C. parvum are being used for indepth studies of modes of action of immunopotentiators on two tumor systems: Lewis lung carcinoma and the MCA 2182 fibrosarcoma. Pyran has been shown to enhance primary and secondary IgM and IgG response of mice to sheep erythrocytes in a way that requires T lymphocytes. The leukocyte adherence inhibition assay and tumor cell induced lymphocyte blastogenesis are in use for evaluation of T-cell function. This work will be concluded in the next contracting period.

Significance for Cancer Research (NCP Objective 4,6 Approach 1,3,4,6)
Understanding the mechanism by which immunopotentiators act is necessary for optimum manipulation of immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: 2/21/74
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $76,451

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy: Development of Therapy with Neuraminidase Treated Tumor Cells

Principal Investigator: Richard K. Gershon, M.D.
Name/Address Yale University School of Medicine
Performing 310 Cedar Street
Organization: New Haven, Connecticut 06510

Contract Number: NO1-CB-43865
Starting Date: 11/08/75 Expiration Date: 11/07/76

Goal: A systematic evaluation of various animal tumors for use in testing of therapy with neuraminidase treated tumor cells.

Approach: Tumor models closely resembling human tumors will be established by various well known means. Neuraminidase and other glycosidases will be tested to determine if they have any effect on the capacity of tumor vaccines to effect tumor regression. Dosage and route of administration of such vaccines will be maximized. Nonviable tumor cells and tumor cell fraction vaccines will be evaluated. Combination therapy with vaccine and other modalities will also be pursued.

Progress: Work performed under this contract is contributing significantly to goals within the immunotherapy program relating to understanding mechanisms of action of cell surface modification in immunotherapy. The investigator is one of several who are working with different tumor systems in animal models to evaluate neuraminidase treatment of cells as an immunotherapeutic maneuver.

Significance for Cancer Research (NCP Objective 6 Approach 1)
Preclinical models of immunotherapy are highly important for optimizing human protocols.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Ccancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $55,000 (Terminal year)

ANNUAL REPORT
The Breast Cancer Program Coordinating Branch
and the Breast Cancer Task Force Program
July 1, 1975 - June 30, 1976

The modus operandi of the Breast Cancer Task Force (BCTF) has undergone major changes in the past year. Under the direction of the new Chairman, BCTF, the following changes have occurred: 1) The Chairperson of each of the four Technical Review Committees, Diagnosis, Epidemiology, Experimental Biology and Treatment is now a non-NCI investigator. 2) The Steering Committee, Advisory to the BCTF program, has representatives from all major divisions of the NCI. 3) The committees meet every two months: the first day is devoted to a joint session during which time 10 to 12 principal investigators of contracts present on-going work; on the second day the technical review committees meet individually; the third day is the meeting of the Steering Committee. 4) A special effort is being made to keep the members of the technical review committees informed of on-going research grants related to the field of breast cancer. 5) A group of grantees working in clinical and basic research of breast cancer are invited to attend each of the joint meetings of the BCTF.

The BCTF contract program has consisted of 82 projects in the past year. On June 30, 1976, the projects will approximate 75 for an estimated funding of $8.8 million. Of the 75 contracts, 61 are renewals and the remainder are new projects. Eleven new program projects have been advertised, the new contracts have been selected primarily from the responses to the advertisements. Nineteen contracts have been terminated in FY-1976 and several have been extended without funds to complete their original objectives.

The BCTF continues to focus its sights on determining the etiology of the disease: identifying high risk populations, improving methods of diagnosis—particularly of small lesions, finding new treatment modalities, and preventing the occurrence and recurrence of the disease. To meet these goals the Task Force contract program considers the totality of breast cancer both in women and in the experimental animal through studies related to eight subject areas to be enumerated below. Some contract projects contribute research in more than one category. The categories and the contracts to which they are assigned are listed below by contract number.

I. Genetic, environmental, dietetic and other factors in breast carcinogenesis. Contracts in this area are: a. Role of obesity and diet rich in proteins, fat and cholesterol—33885, 53884, 53883. b. Markers that define high and low risk cancer families, e.g., HLA-antigens in lymphocytes, blood groups, chromosome polymorphism of blood cells, isoenzymes and steroid profiles in blood: 44003, 44004, 44002, 43940, 43973. c. Host factors in the pathogenesis of mammary dysplasia: 2-3865. d. Production rates of steroids and breast cancer: 33902, 43867, 43898, 43897. e. Exogenous factors (non-contraceptive estrogens) with breast cancer: 53969, 53884.

II. Pathological history of mammary gland and risk of neoplastic transformation. Contracts in this area are: a. Correlation between 1) epidemiological data and anatomical and histological parameters of benign and malignant

tumors: 53968, 53854, 63997, 63995; 2) changes in the pattern of superficial villi as indicators of neoplastic transformation: 2-3861. b. Transplantability of atypical lobules in athymic mice: 4-3908. c. Alterations in the pattern of cystic mastopathy to breast cancer: 43871. Characteristics of cystic fluid: 53853. d. Cytology of nipple fluid as marker of breast pathology: 33882.

III. Isolation, cultivation, and characterization of normal and neoplastic mammary cells of epithelial origin. Contracts in this area are: a. Establishment of long-term cultures of epithelial cells of mammary origin: 1) from human milk: 3-3898; 2) from histo-cultures: 23868; 3) from human pleural effusion: 23869. b. Transplantation of human tumors in athymic mice as a tool for therapy: 3-3856. c. Detect and characterize markers which identify mammary epithelial cells, normal and neoplastic: 1) immunological markers: 3-3906, 23871, 6-3983; 2) isoenzyme patterns: 3-3880, 53885; 3) growth responses to fatty acids: 6-3986. d. Nature of serum factors necessary for growth of mammary epithelium: 2-3875.

IV. Intrinsic and extrinsic factors affecting the defenses of the host and growth potential of mammary carcinoma. Contracts in this area are: a. Role of fat pad in mammary pathophysiology: 1) interaction of stromal cells and mammary epithelium: 4-3907; 2) glucosaminoglycans on basal membrane: 5-3903. b. Transfer of chromosomal DNA into neoplastic cells or from neoplastic to susceptible cells: 4-3904, 5-3904. c. Studies of cell kinetics and prediction of growth potential: 43899, 33903, 33861. d. Evaluation of therapeutic regimen in animal tumor model systems: 43914. e. Effect of antigenic material produced by tumor on the host: 1) inhibition of cellular immunity: 3-3905; 2) blocking of lymphocytic activity: 3-3912. f. Role of cAMP and prostaglandins in breast cancer biology: 3-3920, 3-3919, 3-3909, 3-3918. g. Effects on rodent breast carcinomas of phenylalanine ammonia lyase: 4-3906.

V. Metastases: Characterization, marking, measurement and control. Contracts in this area are: a. Capacity of mammary tumors to metastasize: 2-3864, 6-3980. b. Development of macromolecular ligands able to bind and separate plasma membrane components: 3-3908. c. Properties of normal and neoplastic mammary cell plasma membranes: 3-3910, 6-3984. d. Steroid sulfotransferase and estrogen binding protein levels as markers of disseminated metastases: 43900. e. Post-surgical treatment of human breast cancer: 1) patients with metastases in axillary nodes (Stage II): 43917, 23876, 33899, 43990, 53917; 2) patients with distant metastases: 43991, 64000, 53851.

VI. Physical tools in breast cancer diagnosis. Contracts in this area are: a. Relative merits of xeromammography versus conventional mammography: 43993, 44011, 44012. b. Ultrasound mammography: 23872, 64041. c. Electronic mammographic techniques for mass screening and early detection: 33904. d. Thermography: 43869, 35021, 35027. e. Computerized tomographic mammography: 64047.

VII. Dependency and responsiveness of mammary cells to hormones. Contracts in this area are: a. Role of androgens in morphogenesis of mammary gland and hypothalamic-pituitary axis: 3-3883, 3-3907. b. Detection, isolation and quantitation of estrogen and progesterone binding proteins: 1) detection of bind-

ing proteins: 4-3905, 5-3905, 2-3862, 43969, 2-3862, 43990, 64000; 2) correlation with steroid sulfurylation: 43867. c. Effects of hormones on assembly and function of mammary chromatin: 4-3866, 2-3857. d. Relationship between prolactin and mammary tissue: 2-3859, 2-3863, 6-3985. e. Clinical trials with hormone therapy: 53851, 43991, 64000.

VIII. Support to total program. Contracts in this area are: a. Mammary cells and tissue bank: solid tumors and human cell populations: 3-1146. b. Estrogen-binding protein assay: 43867, 2-3862. c. Pathology lab for cooperative clinical studies: 80704. d. Breast cancer tissue supply: 23867. e. Breast cancer family resources: 33901.

ACTIVITIES

February 10-12, 1975:	"Annual Program Review Conference, Breast Cancer Task Force" (San Antonio, Texas).
July 8, 1975 and May 18, 1976:	BCTF Program Review for the members of the NCAB Subcommittee of the National Organ Site Programs.

PUBLICATIONS

Berlin, N. I. and Vollmer, E. P. (Eds.): Program of the Breast Cancer Task Force. Bethesda, Md. February 1975

Gullino, P. M., Taylor, D. J., and Levine, B. S.: Breast Cancer Task Force Program and Related Projects. Bethesda, Md. January 1976

CONTRACT RESEARCH SUMMARY

Title: Ultrasound Mammography

Principal Investigator: Gilbert Baum, M.D.
Name/Address Performing Organization: Albert Einstein College of Medicine
Bronx, New York 10461

Contract Number: NO1-CB-23872
Starting Date: 6/20/72 Expiration Date: 5/14/76

Goal: To develop the criteria for detection and for the differential diagnosis of breast cancer employing ultrasound, a non-invasive, painless and harmless modality.

Approach: Patients who are to undergo breast surgery are referred into the study on a random basis from breast clinics and surgeons. Scanning is performed on each breast, where the unaffected breast is used as the control. Scanning is begun at the top of the breast and continues down in vertical 3 mm. steps. The ultrasonograms are recorded on 35 mm. half frame film (black and white) and the films from both breasts are simultaneously projected for comparison. Additional film analyses are conducted using an isophotodensitometer, which converts the grey scale of the films into specific colors, in which each color indicates a specific range of amplitude. Finally the ultrasound, clinical and x-ray diagnoses are compared against the pathological report and statistical evaluations are conducted.

Progress: A new ultrasonoscope has been completed and is being tried on patients to establish an examination base level for studying patient unknowns. The new scanner possesses additional scanner motions and a totally new electronic signal system. Initial patient scans show that the new system overcomes the artifacts produced by sector scanning. The ultrasound mammograms of normal and diseased breasts produced by the new unit are being compared with the mammograms produced by the earlier generation ultrasonoscope to establish a new set of diagnostic criteria.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 4/21/76
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast CAncer Steering Committee
FY76 Funds: $186,000

CONTRACT RESEARCH SUMMARY

Title: Purification of an Antigen from Human Mammary Carcinoma

Principal Investigator: Donald M. Marcus, M.D.
Name/Address Performing Organization: Albert Einstein College of Medicine
Bronx, New York 10461

Contract Number: NO1-CB-23871
Starting Date: 06/20/72 Expiration Date: 06/18/77

Goal: (1) To identify antigens associated with breast cancer and to develop a quantitative radioimmunoassay (RIA) for use in immunodiagnosis of breast cancer; (2) to study the correlation between in vitro assays for cell mediated immunity to breast tumors and the clinical status and course of patients, and to identify antigens that elicit cellular immunity.

Approach: (1) to develop antisera specific for tumor-associated antigens, purify the antigens by immunoadsorbent and biochemical techniques and to use the purified antigens in a double-antibody RIA. (2) To make extracts of primary breast tumors obtained at the time of surgical resection and to test autologous and allogeneic extracts against the lymphocytes of the patient by three assays: (a) a viral plaque assay developed by Dr. B. Bloom; (b) inhibition of leucocyte migration; and (c) incorporation of ^{3}H-thymidine.

Progress: (1) An antigen detected in breast tumors was purified and identified as ferritin. A double-antibody RIA for ferritin in human serum has been developed, and it has been found that serum ferritin levels were elevated preoperatively in 41% (14/38) of patients undergoing surgery for a malignant lesion, and in 67% (65/97) patients with recurrent or metastatic breast tumors. We have also detected acidic isoferritins that appear to have antigenic determinants that differ from normal adult liver and spleen ferritins. (2) Extracts of breast tumors prepared with 3M KCl gave positive LMI tests in 11/20 patients with breast cancer and 3/22 controls and individuals with other diseases. An extract of a medullary carcinoma was fractionated by gel filtration on a column of Sephadex G-200. Fraction I (high molecular weight) elicited LMI in 12/22 patients with breast cancer and 1/6 with benign breast disease, whereas Fraction III elicited LMI in 8/23 breast cancer patients and 4/6 with benign disease.

Significanc for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: K. Robert McIntire, M.D.
Program: Breast Cancer Diagnosis Site Visit Date: 2/5/76
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: Approximately $104,000

CONTRACT RESEARCH SUMMARY

Title: Steroid Sulfate Levels as Diagnostic Parameters in Breast Cancer

Principal Investigator: Evan C. Horning, Ph.D.
Name/Address Baylor College of Medicine
Performing Houston, Texas 77025
Organization:

Contract Number: NO1-CB-43897
Starting Date: 01/01/74 Expiration Date: 06/30/76

Goal: To make a detailed comparison of steroid metabolites in urine in a search for possible diagnostic markers for breast cancer.

Approach: Urinary steroid metabolic profiles will be determined using gas chromatographic-mass spectrometric techniques on a group of 25 patients with breast cancer and compared with similar profiles for normal controls and for patients with benign lesions. These studies will be carried out with two 24-hour urine collections for postmenopausal breast cancer patients, postmenopausal patients with benign lesions, and normal postmenopausal subjects. Comparisons of profiles will also be made with premenopausal patients with benign lesions, normal premenopausal subjects, and normal males. This will determine if specific characteristics of steroid metabolism can be associated with breast cancer. Enzymic hydrolysis and chemical solvolysis methods will be used. This work also involves a search for trace amounts of steroidal epoxides, and for possible precursors and metabolic products by means of gas phase analytical methods. The hypothesis is that steroidal epoxides may be involved in breast cancer.

Progress: Comparisons have been made of urinary steroid profiles of postmenopausal patients with breast cancer, with benign breast lesions, normal subjects, premenopausal patients with benign breast lesions, normal subjects and normal males. Three types of profiles have been defined. Type A was found for about two-thirds of the normal females: type B was found for males and about one-third of the normal females: type C was found only for postmenopausal patients with breast cancer or benign breast lesions; some patients also were found to have A and B profiles. It is believed that the patterns of liver steroid reductases are determined by neonatal hormone action, and that type C indicates high risk of postmenopausal breast cancer or benign breast lesions. Comparisons of sulfate and glucuronide conjugates are in progress to determine if sulfate conjugation is affected in patients.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 11/19/73
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $110,000

CONTRACT RESEARCH SUMMARY

Title: Xerommography vs. Film Mammography - A Comparative Study

Principal Investigator: Feargus O'Foghludha, Ph.D.
Name/Address Duke University
Performing Durham, North Carolina 27710
Organization:

Contract Number: NO1-CB-44011
Starting Date: 6/28/74 Expiration Date: 6/26/77

Goal: To evaluate the relative merit of conventional radiography vs. xeromammography in the detection of breast cancer and to delineate differences which may suggest the use of one method over the other in certain situations.

Approach: It is proposed to assess the mammographic image formation and information-transmitting characteristics of each process. Such factors as radiographic contrast, latitude, sensitivity, and modulation transfer function affect the ultimate transmittal of information. Methods to assess these factors will be used to determine optimum techniques in both types of radiography. Specifically, the Duke staff will provide spectrometric services such that the two modalities can be compared under known spectral conditions. Preliminary measurements will be carried out at the Image Research Center at the University of Chicago under subcontract; these will include modulation transfer function for the baseline film mammographic recording medium and the screen film mammographic system manufactured by DuPont. Since the subjective evaluation of the individual examinations should not depend entirely upon the principal investigators as individual bias will intrude, the cephalocaudal films from all institutions will be sent to the Diagnostic Radiology Committee of the National Cancer Institute for review by the advisory board.

Progress: PHYSICS: The photon chopping system has been modified so that constant-potential, full-wave-rectified and other voltage waveforms with varying degrees of ripple can be simulated. To find the charge crossing the tube during the "open" part of the choppint cycle, a light-emitting diode is inserted in the anode circuit. Detector efficiencies and line shapes have been determined by using (a) fluorescence radiations and (b) a crystal-optics monochromator. Over one hundred spectra have been obtained. CLINICAL: Installation of new equipment needed for modifications required by site visitors has been completed and images are now being made by protocol: xerox recording with a) W tube with 0.7 mm Al inherent filter, b) M tube with 0.5 Al filter and c) M tube with Lexan filter.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 4/14/76
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: Approximately $77,000

CONTRACT RESEARCH SUMMARY

Title: Xeromammography vs. Film Mammography - A Comparative Study

Principal Investigator: Philip Strax, M.D.
Name/Address Guttman Institute
Performing New York, New York 10028
Organization:

Contract Number: NO1-CB-44012
Starting Date: 06/28/74 Expiration Date: 06/26/77

Goal: To evaluate the relative merit of conventional radiography vs. xeromammography in the detection of breast cancer and to delineate differences which may suggest the use of one method over the other in certain situations.

Approach: Characteristics of each process are being assessed by way of radiographic contrast, latitude, sensitivity and modulation transfer function.

Progress: 500 women entered into the study in the first year. In the second year, moly filter was used with the Xerox film instead of the aluminum filter. One hundred and forty-two such sets have been made toward the 500 expected. Radiation outputs at M. D. Anderson Hospital have been obtained (air measurements: R/100 MAs). A Victoreen 415 A intercomparison chamber was used with an electrometer and digital read out. 415A was recalibrated at Anderson in terms of R/Coul. Measurements were taken for tungsten tube (no added filtration), and a moly tube (0.03 mm Mo. added) for the AA, Low Dose and xeroradiographic techniques. Thermoluminescent dosimetry in the Low energy range for 0.1 to 25 R. is in progress, so that skin entrance dose measurements may be accomplished.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 4/14/76
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: Approximately $60,494

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Thermography in Mass Screening for Breast Cancer

Principal Investigator: Raymond Fink, Ph.D.
Name/Address Performing Organization: Health Insurance Plan of Greater New York, New York, New York 10022

Contract No.: NO1-CN-35021
Starting Date: June 28, 1973 Expiration Date: 4/17/76

Goal: To evaluate the contribution thermography may make in increasing the effectiveness and/or efficiency of breast cancer screening programs.

Approach: 20,000 women will be screened by three methods: clinical examination, thermography and mammography, each modality being evaluated independently. To obtain the study population, a random sample of all women aged 45-65 belonging to specified medical groups of HIP will be selected. All findings will be reviewed by a medical coordinator who will be responsible for follow-up medical recommendations. This project is being run in conjunction with Thomas Jefferson University, NO1-CN-35027.

Progress: Through November 1975 - 16,1974 women have had initial screening examinations and 3,889 have had recall examinations as a result of findings on the initial examinations. The response rate for those invited to participate in this study is approximately 43 percent. As of June 1975, 67.5 percent of those screened were negative on all three modalities, while 32.5 percent had findings that indicate further follow-up is necessary. Almost 90 percent of these were scheduled for 6-month recall, while the remainder had recommendations for biopsy or aspiration. Biopsy reports were received for 189 patients, with 43 being diagnosed with cancer. Of these 43 patients with breast cancer, only 28 percent had positive thermograms, while 49 percent and 79 percent, respectively, had positive findings on mammographic and clinical examinations. Of the 43 cancers, 38 were diagnosed after the initial screening and 5 were diagnosed after a recall examination. Of the 5 diagnosed after a recall examination, two had negative thermograms on the initial examination. Also of interest is the fact that 40 percent of those with findings on initial examinations and requiring an early recall, were negative on all three modalities on the recall examination.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Harvey Geller Site Visit Date: 3/24/75
Program: Cancer Control
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $ 519,000
Total Funds: 1,630,000

CONTRACT RESEARCH SUMMARY

Title: Tissue Culture Lines from Patients with Breast Cancer

Principal Investigator: Etienne Lasfargues, M.D.
Name/Address Performing Organization: Institute for Medical Research
Camden, New Jersey 08103

Contract Number: NO1-CB-23868
Starting Date: 06/01/72 Expiration Date: 11/30/75

Goal: (1) To develop techniques for the reliable isolation of human breast cancer cells from solid tumors and to stimulate their replication in tissue culture; (2) To supply these cells for use in immunodiagnostic assays.

Approach: Tumor cells are obtained from fresh biopsy specimens selected by a responsible pathologist. The primary cells are seeded in various culture media and supplements previously tested for their ability to support the viability and growth of a standard human breast tumor cell line: BT-20. The neoplastic nature of the isolated cells is determined by Pap's smears, and by their structural organization in sponge matrix cultures as compared with the original histology sections. The cell type is established by electron microscopy of cell contacts and general fine structure.

Progress: Techniques for the isolation of breast carcinoma cells have been perfected and are successful with about 75% of the scirrhous carcinomas. The elimination of fibroblasts is successfully achieved with the judicious use of our serum substitute. Cultures of primary tumor epithelium can be maintained as long as a year in a medium improved with extra glucose, amino acids and vitamins. Mitotic stimulation is obtained with a medium conditioned by human embryonic cells. Cell build-up appears to depend, however, on a competitive ratio of cGMP and cAMP which is presently being investigated.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)
Tissue cultures of tumor cells are essential for development of cell-mediated cytotoxicity assays, which have potential usefulness in the immunodiagnosis of cancer.

Project Officer: Dr. Chou-Chik Ting
Program: Breast Cancer Diagnosis Site Visit Date: 2/28/74
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $33,000

CONTRACT RESEARCH SUMMARY

Title: Anti-uterotropic Assay Data and Progestational and Anti-progestational Assays

Principal Investigator: Marcus M. Mason, D.V.M.
Name/Address Performing Organization: Mason Research Institute
Worcester, Massachusetts 01608

Contract Number: N01-CB-44010
Starting Date: 6/17/74 Expiration Date: 8/31/75

Goal: The compilation and tabular presentation of previously acquired hormone bioassay data with appropriate cross indexing for ease of information retrieval.

Approach: Anti-uterotropic assays-the results of 3,800 assays will be tabulated and cross-indexed.

Progestational assays-the data from 800 assays will be presented in tabular form and an index of activity as compared with the standard will be developed for each assay.

Anti-progestational assays-the data from 500 assays will be presented in tabular form along with an index of activity.

Progress: 1. A format for presentation of the tabular data has been drawn up and presented for approval.

2. Cross indexing will include:
   - NSC Numbers
   - Molecular Formula
   - Alphabetical Name
   - Structural Grouping

The manner in which the cross-indexing will be presented is being prepared now.

Significance for Cancer Research (NCP Objective Approach)
The correlation between chemical structure and biological activity is very important to help investigators choose suitable chemotherapeutic candidates.

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 6/27/74
Technical Review Group: N/A
Relevance Review Group: N/A
FY76 Funds: $3,576 (terminal)

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of Computerized Tomographic Mammography

Principal Investigator: John J. Gisvold, M.D.
Name/Address Mayo Foundation
Performing Rochester, Minnesota 55901
Organization:

Contract Number:
Starting Date: Expiration Date:

Goal: To clinically evaluate the ability of computerized tomographic mammography (CTM) to detect early breast abnormalities and to compare its effectiveness with physical examination, mammography and thermography in individual differential diagnosis.

Approach: A prototype computerized tomographic machine specifically designed to examine the breast has been built by GE and is now in a functional state at Mayo. Six-hundred patients who are scheduled for breast biopsy and who have had mammography and thermography will comprise the study population which will determine if this is a feasible diagnostic examination technique for detection of early breast cancer and at a lower radiation dosage than is used for current mammographic techniques. Later, patients will be selected on a random basis to evaluate the potential mass screening capability of CTM.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich
Program: Breast Cancer Diagnosis Site Visit Date: 11/17/75
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $135,000

CONTRACT RESEARCH SUMMARY

Title: Breast Diagnosis: Quantitative Imaging by Ultrasound

Principal Investigator: James F. Greenleaf, Ph.D.
Name/Address Mayo Foundation
Performing Rochester, Minnesota 55901
Organization:

Contract Number:
Starting Date: Expiration Date:

Goal: To determine if images representing quantitative distribution of basic mechanical properties or values of acoustic parameters of tissue such as velocity, attenuation, reflection, impedance and scattering can be useful in breast cancer diagnosis and of more value than images representing tissue interfaces and geometrics such as B-scans.

Approach: A radial scanner system will be designed and constructed which should acquire ultrasound data from excised tissues by transverse scanning in several modes including echoes for compound B-scans, time-of-flight and attenuation data for mathematical reconstruction and synthetic focus imaging. The ability of acoustic tissue characterization to detect and distinguish excised normal and diseased tissue will be studied both by visual evaluation of the various acoustic images and by computer aided feature extraction and pattern recognition. The resulting images will be compared to the histologic characteristics of the tissue obtained from microscopic evaluation of the section removed from the intact organ. Study will also be begun of normal and diseased breasts in vivo in selected patients and a computer and visual evaluation of patient scan data conducted.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 11/17/75
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $185,450

CONTRACT RESEARCH SUMMARY

Title: Tissue Culture Lines from Patients with Breast Cancer

Principal Investigator:
Name/Address
Performing
Organization:

George Blumenschein, M.D.
Relda Cailleau, Ph.D.
M.D. Anderson Hospital &
Tumor Institute
University of Texas
Houston, Texas 77025

Contract Number: NO1-CB-23869
Starting Date: 06/01/72 Expiration Date: 08/31/76

Goal: To establish tumor cell lines from solid tumors or pleural effusions from breast cancer patients. To supply these cells, effusions and/or blood samples to collaborators for immunodiagnostic assays or other studies. To characterize the lines we have established by chromosomal, biochemical or immunological methods.

Approach: Cells from fresh pleural effusions are concentrated by centrifugation, hemolyzed, neutralized, and resuspended in various media. Aliquots are initially taken for chromosome analysis, staining and electron microscopic study. Primary cultures and established cell lines are frozen in glycerin or DMSO at -70° or in liquid nitrogen for storage or later distribution.

Progress: While no cell lines have been grown successfully from solid primary breast carcinoma, eleven lines have been established from metastases. Ten lines are from pleural effusions and one line from a brain metastasis. Three other cultures have been growing slowly for 2-6 months and may become established lines. The abnormal extra-long chromosomes present in a majority of our lines show a trend that may indicate fusion between specific chromosomes. Inoculation of our cells into nude mice (Dr. Giovanella) has yielded 5 out of 6 positive tumors, whose characteristics resemble that of the original tumor. All tests for G6PD in this laboratory (confirmed and expanded by Dr. Fogh) show our lines to be Type B. Preliminary tests for estrogen binding indicate one (MDA-MB-134) of three lines tested (MDA-MB 157, 231) is positive. Immunological studies using our most prolific lines (231, 157) show antigenic properties both in humoral and cell mediated assay. Cells from 8 of our lines have been frozen to give 10^{8} cells, enough for Dr. Herberman's studies when requested. Several of our lines have been distributed to various laboratories here and abroad by ourselves, Dr. Herberman, Dr. Fogh and Dr. Jensen of the Breast Tumor Cell Bank.

Significance for Cancer Research (NCP Objective 5 Approach 5,6)

Project Officer: Ronald B. Herberman, M.D.
Program: Breast Cancer Diagnosis Site Visit Date: 7/22/75
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $120,000

CONTRACT RESEARCH SUMMARY

Title: Xeromammography vs. Film Mammography - A Comparative Study

Principal Investigator:
Name/Address
Performing
Organization: Gerald Dodd, M.D.
M.D. Anderson Hospital
Houston, Texas 77025

Contract Number: NO1-CB-43993
Starting Date: 06/28/74 Expiration Date: 06/26/77

Goal: To evaluate the relative merit of conventional radiography vs. xeromammography in the detection of breast cancer and to delineate differences which may suggest the use of one method over the other in certain situations.

Approach: This project is being carried out among three institutions, University of Texas, M.D. Anderson Hospital, Duke University Medical Center and Stella and Charles Guttman Breast Diagnostic Institute. Mammographic image formation and information transmitting characteristics of each process will be assessed by way of radiographic contrast, latitude, sensitivity and modulation transfer function. An advisory and monitoring body set up by the Diagnostic Radiology Committee of NCI will review the technical quality of the work performed by the participating institutions. Five-hundred patients per year over 40 years of age will be studied by each institution.

Progress: In the second year of the contracting period at M. D. Anderson Hospital, the clinical protocol has been changed to examining all women included in the study on an overhead tungsten anode mammographic tube with three image receptors: Kodak AA non-screen film, DuPont Lo Dose screen film, and xeroradiographic plates. Since the protocol change approximately 200 women have been entered in the contract and the images are presently being reviewed and correlated statistically with objective parameter measurements derived in the physical aspects of the contract. A modification of the phantom used in the first year of the contract has been completed and images have been obtained and are being analyzed for information content. Objective measurements have been completed save for the specification of the MTF for the image receptors.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 4/14/76
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: Approximately $75,000

CONTRACT RESEARCH SUMMARY

Title: Breast Cancer Tissue Supply

Principal Investigator: Paul O'Brien, M.D.
Name/Address Medical University of South Carolina
Performing Charleston, South Carolina 29401

Contract Number: NO1-CB-23867
Starting Date: 06/30/72 Expiration Date: 06/28/77

Goal: To obtain tissue and blood specimens from patients with breast cancer, for use in immunodiagnostic assays.

Approach: At the time of surgery, obtain tumor and normal breast tissue. Obtain serum and peripheral blood lymphocytes before surgical therapy and at regular intervals thereafter. These specimens are shipped promptly to other investigators in the program for use in immunodiagnostic assays.

Progress: The contract originated in 1972, and since that time more than the minimum number of blood and tissue specimens have been obtained and shipped every year. During the period from March 1, 1975 to March 1, 1976, there were approximately 65 pre-operative blood specimens from patients with malignant breast tumors, 74 malignant breast tumors, 89 blood samples from patients with benign breast disease, 13 cancer of the breast patients skin tested, 93 follow-up blood specimens on patients with malignant tumors and 179 blood samples from patients sent for special studies on patients with breast cancer.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Ronald Herberman, M.D.
Program: Breast Cancer Diagnosis Site Visit Date: 5/72
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $85,000

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Serum LDH Isoenzymes in Women with Breast Tumors

Principal Investigator: Ervin J. Hawrylewicz
Name/Address Mercy Hospital and Medical Center
Performing Chicago, Illinois
Organization:

Contract Number: N01-CB-53855
Starting Date: 06/30/75 Expiration Date: 06/28/77

Goal: To determine if a highly reproducible LDH isoenzyme test can be utilized as a diagnostic marker for breast cancer.

Approach: Blood will be drawn on 800 patients with breast tumors, both malignant and benign, and patients with other gynecological problems. The majority of samples will be obtained prior to surgery. Due to the coding procedures, some past surgical samples will be evaluated. In addition to these patients, blood from 200 women clinically free of breast disease will be obtained as control. All samples will be coded immediately; laboratory personnel will not have access to the code. Serum LDH isoenzyme patterns will be resolved and evaluated. Serums will be classified as Ca Type or N Type (normal). The latter including normal, benign breast tumor, breast disease, other gynecological problems and breast cancer in remission (post-surgical). After submission of evaluations, the data will be compared with the tumor classification to assess the correctness of diagnosis and to determine if other correlations can be made with relationship to e.g., nodal involvement, metastases, cell type, etc.

Progress: In excess of 500 blood samples have been collected, electrophoresed, and densitometry completed. All samples are processed minimally as duplicate determinations utilizing 10 ul serum. Verification determinations require 15 ul of serum. Based on currently established ratios (i.e., LDH 5/LDH 4 and LDH 5+4/LDH 1+2) and related criteria, preliminary diagnosis on 350 samples has been com-leted and released for correlative pathology evaluation.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Ronald Herberman, M.D. and Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date:
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: Approximately $30,000

CONTRACT RESEARCH SUMMARY

Title: Steroid Excretion Study in Patients with Breast Cancer

Principal Investigator: Per Vestergaard, M.D.
Name/Address Performing Organization: Research Foundation for Mental Hygiene
Orangeburg, New York 10962

Contract Number: N01-CB-71465
Starting Date: 06/28/67 Expiration Date: 06/27/76

Goal: To develop automated analysis for determination of urinary steroid hormones.

Approach: The analysis of steroid hormones is based on column chromatography, the column consisting of fine, capillary teflon tubings kept in a constant temperature bath. After the urine extracts are deposited at the head of the column, automation takes over so that all steps become highly reproducible. Even though the analysis per se is nonspecific, the high number of fractions collected allows for fine separation of metabolites without cross contamination.

Progress: The special instrumentation developed under the contract has been described in a series of papers accepted for publication. A monograph describing the steroid methodology is in preparation. Data from the steroid hormone studies in patients and controls are being evaluated. Final evaluation for publication is now being undertaken.

Significance for Cancer Research (NCP Objective 5 Approach 5,4)

Project Officer: Ihor Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 3/28/72
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: None

CONTRACT RESEARCH SUMMARY

Title: Pathology Laboratory for Cooperative Clinical Studies

Principal Investigator: Gilbert Friedell, M.D.
Name/Address St. Vincent Hospital
Performing Worcester, Massachusetts 01610

Contract Number: N01-CB-80704
Starting Date: 03/18/68 Expiration Date: 04/17/77

Goal: To continue the operation of a central pathology laboratory as part of the Primary Therapy of Breast Cancer Study.

Approach: The objective of the above study is to find a correlation between clinical, pathologic and urinary steroid findings which would serve as prognostic indices in women with breast cancer. The central pathology laboratory was established for this study to achieve a standardization of sampling and preparative procedures for histopathologic studies and uniform systematic evaluation. Clinical material is being provided by the members of the Cooperative Breast Cancer Group.

Progress: The initial pathologic studies aimed at assessing the presence of metastases in regional lymph nodes, the presence of blood cell invasion and nuclear grade at the primary tumor site. The data is now being analyzed for correlation with clinical status and urinary steroid findings.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date:
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: None

CONTRACT RESEARCH SUMMARY

Title: Biochemical Analysis of Human Breast Cyst Fluid

Principal Investigator: Morton K. Schwartz, Ph.D.
Name/Address Performing Organization: Sloan-Kettering Institute for Cancer Research
410 East 68th Street
New York, New York 10021

Contract Number: N01-CB-53853
Starting Date: 06/30/75 Expiration Date: 09/15/76

Goal: To search for a diagnostic breast cancer marker in human breast cyst fluid using biochemical and immunochemical techniques.

Approach: 150 breast cyst fluid specimens will be analyzed for cortisol, the androgens, estrogens, progesterone, FSH, LH and prolactin. In addition, tumor associated antigens, minerals, and enzymes will be studied. The objectives will be to determine if the biochemical analysis of human breast cyst fluid can predict those patients who already have or will subsequently develop breast cancer.

Progress: Detailed analysis has been carried out on breast cyst fluid. The material contains remarkably high concentrations of potassium, uric acid and cholesterol. In addition the fluid contains concentrations of carcinoembryonic antigen tissue polypeptide antigen and β-subunit of chorionic gonadotropin that are 20 to 100 fold the concentrations observed in serum or plasma. α-feto protein is not detected. The material contains unusally high concentrations of copper and zinc and high activities of enzymes such as γ-glutamyl transpeptidase, β- glucuronidase and phosphohexose isomerase, but low activities of lactic dehydrogenase, and alkaline phosphatase. Perhaps the most unusual finding are the very marked elevations of androgens. Cortisol, follicle stimulating hormone, prolactin and lutenizing hormone are found in concentrations similar to those in normal plasma. The fluid appears to contain material both of a secretory nature and that resulting from cell breakdown.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D., Quentin Blackwell, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 1/8/76
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: Approximately $200,000

CONTRACT RESEARCH SUMMARY

Title: Primary Breast Cancer Study Patient Input (Follow-up)

Principal Investigator: Anne C. Carter, M.D.
Name/Address Performing Organization: Downstate Medical Center (SUNY) Brooklyn, New York

Contract Number: NO1-CB-23231
Starting Date: 04/16/72 Expiration Date: 12/15/76

Goal: To follow up clinical cases entered in the Primary Breast Cancer Study.

Approach: Primary Breast Cancer Study is aimed at the development of a predictive index for early recurrences after radical mastectomy. One of the more important aspects of the study is the input of eligible patients who are to be followed up for 2 years in regard to possible recurrence of the disease. An essential part of follow-up is the study of laboratory findings and x-rays taken for a minimum of two years to determine recurrences.

Progress: About 70 patients were to be entered by the contractor in the first contract year. After patient accrual had terminated, a follow-up period of two years was instituted. Fifty-six patients were entered on the study. To date, 14 have had recurrence in less than 2 years, 9 have died, 33 are free of recurrence at 2 years and are being followed. After 2 years, patients are to be followed at 6 month intervals until recurrence. Survival data is to be obtained on all patients.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 6/08/72
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: None

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Thermography in Mass Screening for Breast Cancer

Principal Investigator: Jack Edeiken, M.D.
Name/Address Thomas Jefferson University
Performing Philadelphia, Pennsylvania
Organization:

Contract Number: NO1-CN-35027
Starting Date: 6/28/73 Expiration Date: 6/29/76

Goal: To evaluate the contribution thermography may make in increasing the effectiveness and/or efficiency of breast cancer screening programs.

Approach: 20,000 women will be screened by 3 methods: clinical examination, thermography, and xerography - each modality being evaluated independently. To obtain the study population, women aged 45-65 from a variety of sources (American Cancer Society screenees, Bell Telephone Company employees, etc.) will be invited to participate. All findings will be reviewed by a medical coordinator who will be responsible for follow-up medical recommendations. This project is being run in conjunction with the Health Insurance Plan of Greater New York, NO1-CN-35021.

Progress: Through December 31, 1975 - 16,754 patients have been screened. This is a predominantly white population (89 percent) with more than half (58 percent) under age 55. Of 14,507 for whom results were available, 3,214 or 22 percent had findings requiring 6-month follow-up, and 561 or 4 percent were referred for biopsy. Of these 561 patients, 408 had a diagnostic workup including biopsy, and 138 or 34 percent were diagnosed with a malignancy. Thermography was positive in 41 percent of those diagnosed with cancer, while 75 percent were positive on mammography and 57 percent had positive clinical findings. The 138 cases diagnosed with malignancy resulted in a case rate of 8.6 per 100,000 population, significantly higher than expected.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Harvey Geller Site Visit Date: 3/25/75
Program: Cancer Control
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $ 997,400
Total Funds: 2,253,300

CONTRACT RESEARCH SUMMARY

Title: Analysis of Breast Secretions

Principal Investigator: Nicholas L. Petrakis, M.D.
Name/Address Performing Organization: University of California Medical Center
Hooper Foundation
San Francisco, California 94143

Contract Number: NO1-CB-33882
Starting Date: 5/15/73 Expiration Date: 5/13/77

Goal: To evaluate Sartorius Breast pump technique as a means of breast cancer detection, using cytological, biochemical and virological analysis of secreted material.

Approach: Several studies are being pursued: (a) correlation of cytologic findings on nipple aspirates in women with undiagnosed breast masses and comparison with mammography and surgical pathology. Attempts are being made to find tumor cells, cellular atypia, and/or specific biochemical changes, (b) aspirations from women attending the ACS-NCI Breast Cancer Demonstration Center at Merritt Hospital in Oakland. Studies have included initial cytological examination and, if sufficient fluid was available, RNA-dependent DNA polymerase protein electrophoresis, immunoglobulins, IgA, IgG, IgM, peroxidated lipid and other constituents.

Progress: (a) A detailed analysis has been made, of the 1779 breasts examined cytologically. Abnormal cells were found in fluids from 197, of which tissue was available in 42. Among these 42 with tissue, 14 were breast cancer and 28 were lesions in the spectrum of so-called mammary dysplasia. Among the remaining 1582 breasts with benign cytology, tissue was available for comparison in 80, of which 9 had breast cancer and 71 benign breast lesions in the mammary dysplasia complex. The ultimate role of cytology will depend upon the final outcome in those women with cellular abnormalities and no clinical disease. In the first 2636 breasts examined, there were 87 in this category. This group deserves especially close attention to follow-up procedures over a satisfactory length of time. (b) A striking increase (20X) in IgM was found in breast fluids of breast cancer and mastectomy women as compared to normal and benign disease. These findings suggest that IgM may have diagnostic and prognostic importance in abnormal cytology and breast cancer screening programs. Prospective studies are in progress.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 3/4/76
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: Approximately $87,646

CONTRACT RESEARCH SUMMARY

Title: Screening for Breast CAncer by Electronic Infrared Pattern Recognition

Principal Investigator: JoAnn D. Haberman, M.D.
Name/Address University of Oklahoma Health
Performing Sciences Center
Organization Oklahoma City, Oklahoma 73190

Contract Number: NO1-CB-43869
Starting Date: April 1, 1974 Expiration Date: Sept. 30, 1976

Goal: To design and clinically test a prototype system for mass thermographic screening of breast cancer and douple it with computer processing for automated evaluation.

Approach: This project is phase 1 of a planned 3-phase development program. The first phase would include: (1) construction of an improved instrument utilizing the uniqu serial scan multiple element developed by HOneywell; the instrument will be equipped for direct recording on a standard video recorder and conventional television display; (2) a program of clinical evaluation to assess the utility of the various features in the diagnosis of breast cancer and compare with results of other modalities; (3) definition of the system specifications for a mass screening prototype instrument, and (4) development of initial data base for automated screening evaluation.

Progress: Clinical evaluations are proceeding on a group of asymptomatic subjects. Cooling studies indicate that 15 minutes must be allowed for equilibration time for each subject. Absolute temperatures are manually recorded over regions of each breast and a video tape record is made. The video tape records are being examined and will be digitized and evaluated later in the program. Symptomatic patients scheduled for biopsy are also being evaluated. At the present time data on approximately 20 patients has been recorded.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 2/12/73
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: None

CONTRACT RESEARCH SUMMARY

Title: Electronic Mammography

Principal Investigator: Donald Sashin, Ph.D.
Name/Address University of Pittsburgh
Performing Pittsburgh, Pennsylvania 15260
Organization:

Contract Number: NO1-CB-33904
Starting Date: June 20, 1973 Expiration Date: Sept. 30, 1975

Goal: Develop electronic mammographic technique designed for mass screening for early breast cancer detection. This system has the advantages of rapid low cost operation, small film format, reduced radiation exposure to the patient, selective frequency enhancement, low noise and reduced film storage costs.

Approach: Electronic mammography involves substitution of image intensification and TV techniques for x-ray film, increasing the efficiency with which x-rays can be recorded. The program consists of two parts: (1) design and construction of a prototype system optimized for visualization of soft tissues and (2) evaluation of the system regarding its ability to display clinical details relative to present mammographic systems. In this method a mammographic image display on a intensifying screen is viewed by a high resolution TV camera. The TV image is displayed on a cathode ray tube and photographed automatically by a 70 mm roll film camera.

Progress: The system has been designed and evaluated with phantoms and in the clinical setting with regard to resolution, noise and clinical performance. Each component of the system has been carefully optimized to insure the best final image quality. In the phantom studies the system exceeded 70 mm photofluorographic mammography in resolution while requiring comparable radiation exposure. In the preliminary clinical study, small tumors and other pathology were demonstrated. Modifications have been made to the original system design to further improve performance. These include a moving slit technique and redesign of some of the electronic circuits. Phantom measurements of the system performance with these new improvements show that the radiation dose is reduced and the image noise is lowered while the image contrast is improved by about 100% compared to the earlier performance of this system.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 8/27/74
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $28,000 (Six months terminal)

CONTRACT RESEARCH SUMMARY

Title: Primary Breast Cancer Study Patient Input (Follow-up)

Principal Investigator: Carleton R. Haines, M.D.
Name/Address University of Vermont
Performing Burlington, Vermont
Organization:

Contract Number: NO1-CB-23232
Starting Date: March 13, 1972 Expiration Date: Sept. 12, 1976

Goal: To follow up clinical cases entered in the Primary Breast Cancer Study.

Approach: Primary Breast Cancer Study is aimed at the development of a predictive index for early recurrences after radical mastectomy. One of the more important aspects of the study is the input of eligible patients who are to be followed up for 2 years in regard to possible recurrence of the disease. An essential part of follow-up is the study of laboratory findings and x-rays taken for a minimum of 2 years to determine recurrence.

Progress: Forty-six (46) patients have been entered since June 15, 1972. Twenty-eight of these have received the 3 and 6 month follow-up examination. Nine (9) of these cases were negative, while six (6) of these cases were dropped for various reasons. This is the terminal year of follow-up. Eleven patients will be followed until September 1975 to complete the two-year minimum.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)
Having a prognostic handle on the course of the disease after mastectomy would help in planning further adjuvant/prophylactic therapy.

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 6/09/72
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: None

CONTRACT RESEARCH SUMMARY

Title: Fibrocystic Disease and its Relationship to Breast Cancer

Principal Investigator: David L. Page, M.D.
Name/Address Vanderbilt University
Performing Nashville, Tennessee 37232
Organization:

Contract Number: NO1-CB-43871
Starting Date: January 15, 1974 Expiration Date: January 14, 1977

Goal: To determine which lesion or which combination of lesions, if any, in chronic cystic mastopathy leads to the development of mammary carcinoma.

Approach: The investigator is assembling, reviewing and classifying slides from breast biopsies of 1000 women performed 15 or more years ago and diagnosed as cystic disease at that time. All slides will be classified as to component parts of cystic disease according to the Foote and Stewart classification system. Patients will be followed until their death or the development of mammary carcinoma. Each patient, each patient's record, and physician or family will be contacted to secure the desired information. Correlative studies between the component types of cystic disease (including papillary and lobular proliferative lesions) and the development of mammary carcinoma will be undertaken. An attempt will be made to demonstrate how various kinds of cystic disease lesions are associated with the development of mammary carcinoma.

Progress: 1098 individuals with benign biopsies were accepted for review and categorized by type of cystic disease, following completion of pilot study which tested feasibility of follow-up contact and questionnaire content. 970 letters of inquiry were mailed with questionnaires completed for 801 subjects.

Significance for Cancer Research (NCP Objective 5 Approach 5.2)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date:
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $54,100

CONTRACT RESEARCH SUMMARY

Title: Blood Steroid Sulfate Levels as Diagnostic Parameters in Breast Cancer

Principal Investigator: Federico Welsch, M.D., Ph.D.
Name/Address Performing Organization: Worcester Foundation for Experimental Biology, Inc. Shrewsbury, Massachusetts 01545

Contract Number: NO1-CB-43898
Starting Date: January 1, 1974 Expiration Date: Dec. 31, 1976

Goal: To analyze blood steroid sulfate levels to serve as possible diagnostic and/or prognostic parameters in breast cancer.

Approach: Estrone sulfate, dehydroepiandrosterone sulfate and androsterone sulfate will be measured in the plasma of at least 100 patients per group, either with diagnosed breast cancer or diagnosed benign breast tumors or apparently normal controls using radioimmunoassay techniques. These measurements can be used to indicate possible changes in adrenal steroid production in postmenopausal women and adrenal and ovarian activity in young women occurring after the development of breast cancer. It would be an attempt to find a unique metabolite in blood which might be characteristic of breast cancer.

Progress: Seventy samples have been analyzed for estrone sulfate, dehydroepiandrosterone sulfate and androsterone sulfate. Some problems have been encountered with the latter assay and are currently being evaluated. Further samples will be received and analyzed.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Ihor J. Masnyk, Ph.D., Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 2/4/76
Technical Review Group: Breast CAncer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: No Cost Extension

CONTRACT RESEARCH SUMMARY

Title: Automated Methods for Determination of Steroid Metabolites

Principal Investigator: Frederico Welsch, M.D., Ph.D.
Name/Address Performing Organization: The Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts 01545

Contract Number: NO1-CB-60968
Starting Date: June 10, 1966 Expiration Date: June 9, 1976

Goal: To develop automated analysis for determination of urinary steroid hormones.

Approach: This analysis of steroid hormones is based on paper chromatography with uniquely designed triple wavelength readout permitting an automated Allen correction to be incorporated. The methodology is highly specific and reproducible, permitting the analysis of a spectrum of hormonal metabolites on 5-10% aliquot of a 24-hour specimen.

Progress:During the last year 540 urine samples were analyzed. The laboratory serves as the analytical arm of the clinical study designed to develop a predictive index for spotting early recurrences after radical mastectomy. Patient accrual and follow-up has ended, and the contractor is checking out one year postmastectomy values of urinary steroids. The contract is in its terminal phase. The contractor requested and received a one year no cost extension.

Significance for Cancer Research (NCP Objective 6 Approach 5.4)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 1971
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: None

CONTRACT RESEARCH SUMMARY

Title: Epidemiology of Pre- and Post-Menopausal Breast Cancer

Principal Investigator: Baruch Modan, M.D.
Name/Address Performing Organization: Chaim Sheba Medical Center
Tel Hashomer, Israel

Contract Number: NCI-CB-63995
Starting Date: Expiration Date:

Goal: To determine the epidemiologic characteristics of non-invasive breast disease and of both pre- and post-menopausal breast cancer and to compare the three groups.

Approach: Both the study of non-invasive breast disease and the study of pre- and post-menopausal breast cancer will be retrospective case control interview studies, each one of 500 newly diagnosed cases and of 1000 matched controls. In each study there will be two control groups, one drawn from hospitals and one from neighborhoods. The studies will cover both high and low risk Israeli populations. All analyses will be performed separately for ethnic groups, histopathologic category, and by pre- and post-menopausal status. Phase II of the first study will entail a nationwide incidence study of benign breast disease.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 5.1 and 5.2)

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 4/8/76
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: Approximately $40,000

CONTRACT RESEARCH SUMMARY

Title: Breast Cancer Family Resources

Principal Investigator: Henry T. Lynch, M.D.
Name/Address Creighton University
Performing School of Medicine
Organization: Omaha, Nebraska 68178

Contract Number: NO1-CB-33901
Starting Date: June 30, 1973 Expiration Date: June 29, 1977

Goal: To establish a research pool of about 80 well-documented breast cancer-prone families to be used for medical genetic studies.

Approach: There is continuous updating of genealogy and tumor history by personal interviews with patients and physicians and by telephone calls and letters. A critical analysis of these families is continued in a search for basic genetic mechanisms through the study of genealogy and varying combinations of malignant neoplasms as well as other medical disorders in this series of breast cancer-prone families. Pedigrees with detailed descriptions which are compiled on each family are available for review by each contractor who expreses an interest in studying these families. Access to the resource by qualified investigators (with separate funding) is subjected to approval by the contractor in consultation with the Breast Cancer Task Force.

Progress: From the 94 families in the resource 75 families are confirmed as verified breast cancer families. Biological specimens obtained from members of these families are being supplied to various investigators for the following studies: 1) Human Lymphocyte-Antigen (HL-A); 2) Carcinoembryonic Antigen (CEA); 3) Bovine C-type Virus; 4) Viral Antibov and Cytogenetic Studies; 5) Genetic Polymorphisms; 6) Aryl-hydrocarbon Hydroxalase; 7) Virology Studies on Placentas, Tumor Specimens and Leukocytes using electron microscopy; 8) Mamalian C-type RNA Tumor Viruses; 9) SMT-Antigen; 10) Type-C Viruses; 11) Lymphocyte Cytoxicity; 12) Immunological Studies including T & B Lymphocytes and Lymphocyte Alpha-Fetoprotein.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Elizabeth Anderson, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 4/10/75
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $59,500

CONTRACT RESEARCH SUMMARY

Title: Polymorphisms in High and Low Risk Breast Cancer Families

Principal Investigator: Henry T. Lynch, M.D.
Name/Address Creighton University
Performing School of Medicine
Organization: Omaha, Nebraska 68178

Contract Number: N01-CB-44003
Starting Date: June 17, 1974 Expiration Date: June 16, 1977

Goal: To investigate histocompatibility antigens and other genetic polymorphisms in high and low risk breast cancer families.

Approach: HL-A markers, genetic polymorphic markers such as red blood cell enzymes, (acid phosphatase, adenosine deaminase, etc.) serum proteins (haptoglobins a-chain, group-specific component, etc.), and blood group systems (ABO, Duffy, Lewis, MN, etc.) and specific pathologic diagnoses (all histologic varieties of cancer retrospectively in the family context) are being evaluated in approximately 20 multiple cancer case families selected from the total clinical pool of 95 high risk families and kindreds.

Progress: Eighteen of twenty multiple case families have been sampled. A total of 575 individuals have been seen and blood samples were obtained. Specimens have been sent to the investigators, Dr. Terasaki, University of California, Los Angeles, for HL-A typing, and Dr. Petrakis, University of California, San Francisco, for genetic polymorphism studies. Analysis has been completed on all individuals for the following systems: ABO, Duffy A and B, Lewis A and B, MNS, RH (Cc, Dd, Ee), group-specific component, and haptoglobin (a-chain). Analysis for acid phosphatase adenylate kinase, esterase D, and phosphoglucomutase-1 are in progress. Preliminary technical work is in progress on the remaining system: adenosine deaminase, soluble glutamate-pyruvate transaminase, peptidase A, Al-anti-trypsin, and the third complement. The majority of pathology slides and reports, which represent the varieties of cancer diagnosed in all of the breast cancer families, have been forwarded to Dr. Mulcahy, Jersey City Medical Center, for his evaluation.

Significance for Cancer Research (NCP Objective 5 Approach 5.14)

Project Officer: Joseph Fraumeni, M.D., Elizabeth Anderson, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 4/10/75
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $120,000

CONTRACT RESEARCH SUMMARY

Title: A Study on Familiality of Breast Cancer in Iceland

Principal Investigators: Dr. Nicholas E. Day
Name/Address (Dr. H. Tulinius: Co-investigator in Iceland
Performing Organization: International Agency for Research on Cancer
Lyon, France

Contract Number: NO1-CB-43973
Starting Date: June 30, 1973 Expiration Date: June 29, 1977

Goal: To determine whether there are hereditary characteristics which might be of use in the identification of women having a high risk of developing breast cancer.

Approach: The unique facilities available in Iceland for the study will be utilized. These include: (1) a nationwide cancer registry functioning since 1954 (about 800 cases), all cases of breast cancer diagnosed between 1910 and the start of the cancer registry are on file, (2) a computer-based National Roster since 1952, (3) a family linkage file linking every individual alive after 1910 to his parents and children, and (4) a cervical screening clinic which has seen more than 80% of the female population of Iceland born between 1905 and 1945. Information is collected on reproductive history and age at menarche, to be used as control information. If an excess risk is found, an effort will be made to (1) correlate familial risk with recognized epidemiological risk factors for breast cancer, (2) quantify the risk attached to different types of relationships, and (3) for each class of relative, examine the association, if any, between the degree of risk and the age of the proband case and age of appearance of cancer among these relatives.

Progress: Analysis of incidence data from 1911-1972 has shown a marked increase in breast cancer during the period and a corresponding change in the shape of the age incidence curve. This change in shape can be explained solely as a cohort effect. Genealogies of 384 families have been completed, a further 35 are in preparation. The family members are in the process of being linked to the list of cases. Relative risks associated with age at first pregnancy and parity have been calculated, and shown not to change with year of birth. Expected rates of breast cancer among different classes of relative are being calculated. Statistical methods for assessing familial risk with known but differing degrees of ascertainment are being developed.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date:
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $32,000

CONTRACT RESEARCH SUMMARY

Title: Biochemical and Physiological Investigations Based on Familial Genetic Patterns

Principal Investigator: Jack Fishman, Ph.D.
Name/Address Performing Organization: Montefiore Hospital and Medical Center
Bronx, New York 10467

Contract Number: NO1-CB-44002
Starting Date: June 24, 1974 Expiration Date: June 23, 1977

Goal: To evaluate the possible role of genetic factors in the expression of specific biochemical parameters in breast cancer pathophysiology.

Approach: Endocrine assays will be done on blood and urine of premenopausal females from families with a high incidence of breast cancer and from families with a high incidence of breast cancer and from matched control families. Morning blood samples obtained every second day throughout the menstrual cycle will be assayed for FSH, LH E_3, E_2, E_1, progesterone and prolactin. Nocturnal urines (7 P.M. to 7A.M.) will be assayed for 11 to 13 estrogenic, androgenic and corticosteroid metabolites. An attempt will be made to correlate familial susceptibility to breast cancer with endocrine parameters.

Progress: This report covers the period ending March 5, 1976. Plasma and urine samples from 35 women have been received to date from Dr. Lynch. Plasma hormone measurements are now completed or partially complete in 29 subjects. Estriol is now being measured only in pooled follicular and luteal plasma samples since its concentration is too low to determine accurately in individual samples. Urinary steroid hormone measurements are complete or almost complete in 28 of the subjects, and have already been started in another 4. 2-Methoxyestrone has been measured in 7 subjects. The daily variation in this metabolite is quite small so that future measurements will be done on pooled follicular and luteal fractions. On March 4, 1976, a meeting was held with Dr. Henry Lynch and the biostatisticians from Omaha concerning the treatment of the data. Analysis of the data will be performed in consultation with both institutions.

Significance for Cancer Research (NCP Objective Approach)

Project Officer: Elizabeth P. Anderson, Ph.D., Charles Brown, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 3/29/74
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $187,411

CONTRACT RESEARCH SUMMARY

Title: Genetic Polymorphisms in High and Low Risk Breast Cancer Families

Principal Investigator:
Name/Address
Performing
Organization: Frans J. Cleton, M.D.
The Netherlands Cancer Institute
Amsterdam, The Netherlands

Contract Number: NO1-CB-43940
Starting Date: June 18, 1974 Expiration Date: Dec. 17, 1976

Goal: To study linkage of certain genetic markers with breast cancer in an attempt to select high risk groups in the population for the development of breast cancer.

Approach: HL-A haplotypes will be studied in families with a high incidence of breast cancer. A large number of other genetic markers will also be investigated, such as blood groups, immunoglobulins, red cell isoenzymes and chromosomal polymorphisms. As a peripheral project, group specific antigens will be studied, using a sensitive micro-immune diffusion technique with anti-mouse MTV sera and milk of lactating family members will be collected for study of a possible human MTV using electron microscopy and biochemical techniques. Plasma prolactin levels will be measured in all high risk family members. Because there is some indication that the histology of familial breast cancer is different with respect to lymphocytic infiltration from general breast cancer, a histological classification of all tumor biopsies will be carried out.

Progress: Up to September 1975, a total of 14 high-risk families are under investigation. All available members in the first 9 families were typed for HL-A antigens. In the last 5 families investigations will only be carried out in selected members, who will be expected to provide us with relevant information. In 41 members of these 5 families a complete analysis of all the genetic markers mentioned in the program has been done.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Joseph Fraumeni, M.D., Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date:
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $55,000

CONTRACT RESEARCH SUMMARY

Title: An International Study on the Role of Overweight in Breast Cancer Epidemiology

Principal Investigator: Fritz de Waard, M.D.
Name/Address Queen Wilhelmina Fund
Performing Amsterdam, The Netherlands
Organization:

Contract Number: NO1-CB-33885
Starting Date: July 1, 1973 Expiration Date: June 27, 1976

Goal: To produce age-specific incidence curves of breast cancer for various subgroups differing in body weight and height within geographically defined populations.

Approach: Calculate incidence rates for the above mentioned subgroups on the basis of case-control comparisons (relative risk estimates) in regions where a population-based cancer registry operates.

Progress: Data have been collected in 1400 cases and 6000 controls in the cities of Rotterdam and The Hague and the province of Friesland. Collection of data has virtually been completed. In The Hague a sample of the non-responders among controls has been approached again to evaluate bias due to selective response. Preliminary data from Friesland were reported at the Key Biscayne Conference in May 1975. Analysis of the data are now in progress.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 4/4/73
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $12,000

CONTRACT RESEARCH SUMMARY

Title: Epidemiologic Characteristics of Medullary and Lobular Breast Cancer

Principal Investigator:
Name/Address
Performing
Organization: Paul P. Rosen, M.D.
Sloan-Kettering Cancer Center
New York, New York 10021

Contract Number: NCI-CB-63997
Starting Date: Expiration Date:

Goal: To identify and describe morphologic characteristics of medullary, lobular, and related mammary carcinomas which are relevant to known risk factors for the development of such carcinomas.

Approach: Morphologic features of breast carcinoma will be defined in 1200-1500 breast cancer patients over a three-year period, and patients with ductal, medullary, and lobular carcinoma will be identified. Each patient will then be interviewed, and the epidemiologic information gained will be evaluated for (1) possible differences between patients with ductal carcinoma and those with medullary or lobular carcinoma, (2) possible morphologic characteristics associated with familial risk, and (3) morphologic features that are epidemiologically and/or prognostically significant.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 5.6)

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 4/21/76
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: Approximately $80,000

CONTRACT RESEARCH SUMMARY

Title: Possible Correlations Between Dietary Factors and Epidemiological Breast Cancer

Principal Investigator: Baruch Modan, M.D.
Name/Address Performing Organization: Chaim Sheba Medical Center, Tel Hashomer and Tel Aviv University Medical School Tel Hashomer, Israel

Cotract Number: NO1-CB-53883
Starting Date: June 30, 1975 Expiration Date: June 29, 1977

Goal: To carry out epidemiological investigations of selected population groups to elucidate the possible role of diet and/or nutritional status on the incidence, pathogenesis and natural history of breast cancer.

Approach: A case-control study using a validated questionnaire will be conducted to determine whether dietary components particularly fats and (low) cellulose, may serve as risk factors in the etiology of breast cancer. The study population will be composed of 500 newly diagnosed breast cancer cases of which 300 will be European and American born and 200 will be Asian-African born. The 1000 controls will be matched for age, menopausal status, country of origin, period of immigration to Israel. Anthropometric measurements will be included.

Progress: Questionnaire perfected: pre-test completed. 140 cases and 120 controls interviewed thus far.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Elizabeth P. Anderson, Ph.D., Charles Brown, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 4/8-9/76
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $41,225

CONTRACT RESEARCH SUMMARY

Title: Epidemiologic Characteristics of Pre- and Post-Menopausal Breast Cancer

Principal Investigator: Ralph S. Paffenbarger, M.D.
Name/Address University of California
Performing Berkeley, California 94720
Organization:

Contract Number: NCI-CB-63996
Starting Date: Expiration Date:

Goal: To identify host and environmental factors that predispose to the development of breast cancer and to contrast the risk factors for pre- and post-menopausal cancer.

Approach: Using epidemiologic methods of case-control design, structured interviews will be conducted with women of Caucasian, Negro, and Oriental origin, totaling 2500 breast cancer patients and 5000 controls over a three-year period. Known risk factors will be explored as well as extent of disease and histological diagnosis. Amassed data will be processed by appropriate statistical methods to search for associations between epidemiological characteristics and pre-menopausal vs post-menopausal breast cancer.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 5.1 and 5.6)

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 4/15/76
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: Approximately $76,000

CONTRACT RESEARCH SUMMARY

Title: Epidemiology of Non-Invasive Breast Disease

Principal Investigator: Gary H. Spivey, M.D.
Name/Address University of California, Los Angeles
Performing Los Angeles, California 90024
Organization:

Contract Number: NCI-CB-63995
Starting Date: Expiration Date:

Goal: To determine risk factors for each major type of non-invasive breast disease and to compare these with risk factors for breast cancer.

Approach: A case-control study will collect epidemiological information on patients with diagnosed non-invasive breast disease and on two control groups, one drawn from the same medical source and one from friends of the patients. Information will also be collected on a fourth group of breast cancer patients. The data will be analyzed and the risk factors identified as being of etiologic importance for the various histologic types of non-invasive breast disease will be compared, in each case, with those found to be significant for the development of breast cancer.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 5.2)

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 4/14/76
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: Approximately $150,000

CONTRACT RESEARCH SUMMARY

Title: Role of Dietary Factors and Non-Contraceptive Estrogens in Breast Cancer

Principal Investigator: Tomio Hirohata, M.D.
Name/Address University of Hawaii at Manoa
Performing Cancer Center of Hawaii
Organization: Honolulu, Hawaii 96822

Contract Number: NO1-CB-53884
Starting Date: June 30, 1975 Expiration Date: June 29, 1977

Goal: To determine in post-menopausal women if there is an association of breast cancer with the use of non-contraceptive estrogens and with specific dietary factors.

Approach: A case-control approach is being utilized in which a validated questionnaire is employed to collect information on past history of drug usage (including exogenous estrogens) and usual weekly dietary intake. Breast cancer cases from three selected populations (ages 45-74) are identified: (1) the Caucasians in Hawaii who are at high risk for breast cancer; (2) the Japanese in Hawaii who are at intermediate risk; and (3) the Japanese in Fukuoka, Japan representing a low risk population. For each case, a neighborhood and hospital control matched by race and age, is interviewed.

Progress: The pre-test phase of the study has been completed since then. Interviews have been done in Hawaii on 24 sets of triplets, consisting of a breast cancer case and her neighborhood and hospital control. In Japan, interviews have been completed on 20 sets, as of February 10, 1976.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Elizabeth P. Anderson, Ph.D., David Levin, M.D.
Program: Breast Cancer Epidemiology Site Visit Date:
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $115,900

CONTRACT RESEARCH SUMMARY

Title: Possible Correlations between Morphology and Epidemiology of Breast Cancer

Principal Investigator: H. Stephen Gallager, M.D.
Name/Address Performing Organization: The University of Texas System Cancer Center
M.D. Anderson Hospital and Tumor Institute
Houston, Texas 77025

Contract Number: NO1-CB-53854
Starting Date: June 30, 1975 Expiration Date: June 29, 1977

Goal: To demonstrate morphologic markers characteristic of familial types of breast disease in order to identify selected high risk populations.

Approach: A previously developed system for the profiling of the morphologic characteristics of breast cancer which integrates information from gross and microscopic examination, mammography, and specimen radiography and requires the rough quantitative evaluation of some 39 factors in these three groups will be validated and refined. The breast cancer specimens will be derived from three major risk groups which have been characterized genetically. These consist of 114 patients with mothers with cancer pedigress, 97 patients with sisters with cancer pedigrees and 111 patients with second order pediwiees for breast cancer. The findings will be compared with 300 patients without family.

Progress: The planned validation phase of the study has been completed and modifications of the data collection system and evaluative criteria have been completed. A paper is in preparation concerning the biomathematical techniques used in this phase. It is believed that they may have relevance to the development of quality control procedures for diagnostic histopathology. General data recording using the refined system began about December 1, 1975. To date, approximately 65 cases have been completed by all three observers and an additional 40 cases have been completed by one or two observers. Following recommendations of the Site Visit Committee that visited January 28, 1976, arrangements have been made to add to the data to be collected information relative to survival. With this addition, the prognostic significance of the various features studied will also be available.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Elizabeth P. Anderson, Ph.D., Philip Prorok, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 1/28/76
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $44,903

CONTRACT RESEARCH SUMMARY

Title: Genetic Polymorphisms in High and Low Risk Breast Cancer Families

Principal Investigator: David E. Anderson, Ph.D.
Name/Address University of Texas System
Performing Cancer Center
Organization: Houston, Texas 77025

Contract Number: NO1-CB-44004
Starting Date: June 16, 1974 Expiration Date: March 15, 1977

Goal: To investigate a series of polymorphic genetic markers in high and low risk breast cancer families which might assist in the definition of high-risk individuals.

Approach: The ABO, Rh, MNS, P, K, Fy^a and Jk^a blood group types; serum haptoglobin types; alkaline phosphatase and phosphoglucomutase 1 red blood enzyme types; and chromosome abnormalities and/or variants including sister chromatid exchanges will be determined from blood specimens collected from patients and their unaffected sister who belong to high-risk families. These will be compared with women who have no history of breast cancer in themselves or their families and who match the high-risk women in age and menopausal status. The chromosome studies will be conducted on smaller numbers of women in whom the genetic effect is maximized as evidenced by the occurrence of the disease in at least three first-degree relatives.

Progress: Blood specimens from 606 family members have been typed for genetic markers, and specimens have been collected from 78 individuals for chromosome studies. A trend is becoming evident for the ABO blood group system. Patients from high-risk families with premenopausal diagnosis have a high frequency of blood type O (55.9%), while those with postmenopausal diagnosis or those from the moderate-risk families (sister pedigree) whether diagnosis is pre- or post-menopausal, have a high frequency of type A (48.7%). The Rh type shows no trend. The other markers have not been evaluated for the effect of age at diagnosis. A significant finding from the chromosome studies is that the breast cancer patients and their unaffected sisters have an average of 2.6 C-band variants per individual whereas controls have an average of 1.6. However, the number of tested individuals in the chromosome studies is small and larger numbers will be required to enhance the reliability of the results.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Joseph Fraumeni, M.D. and Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 1/28/76
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $43,743 (nine months)

CONTRACT RESEARCH SUMMARY

Title: Biochemical and Physiological Investigations Based on Familial Genetic Patterns

Principal Investigator: David E. Anderson, Ph.D.
Name/Address The University of Texas System
Performing Cancer Center
Organization: Houston, Texas 77025

Contract Number: NO1-CB-43939
Starting Date: June 17, 1974 Expiration Date: Dec. 16, 1975

Goal: To measure aryl hydrocarbon hydroxylase (AHH) inducibility levels in cultured lymphocytes from members of high-and low-risk breast cancer families for possible use as a genetic marker.

Approach: Lymphocytes are isolated from fresh human blood specimens using Ficoll-Hypaque density gradient centrifugation. The cells are cultured in a medium of Eagle's medium and fetal calf serum, with stimulation of mitosis by a mixture of phytohemmaglutinin and pokeweek mitogen. At the end of three days, experimental tubes are induced with a solution of 3-methylcholanthrene in acetone. Control tubes receive an equal volume of acetone. At the end of another 24 hours, the cells are harvested and assayed for AHH activity by a spectrophotofluorometric method using benzypyrene as substrate. Inducibility is expressed as the ratio of the activity of induced cells over the activity of control cells (induced ratio).

Progress: Significant improvements in the lymphocyte culture and AHH assay protocols have been achieved. Those include (1) a 2.5-fold increase in sensitivity of the assay by optimizing the assay pH; (2) close control of the pH of the culture medium by culturing the cells in a 5% CO_2 atmosphere to reduce variations in base level activities; and (3) considerable improvement in cell growth by using tissue culture flasks instead of glass culture tubes. Since repeat cultures from the same subjects are variable, increased reproducibility of the assay will be required to provide meaningful data. Variability of induction ratios appears to be associated primarily with different lots of fetal calf serum in the culture medium. We are currently attempting to reduce this variability by reducing the serum concentration from 20% to 5% in RPMI 1640 culture medium. Reproducibility of the modified protocol is being evaluated using a nucleus of volunteers to provide periodic blood samples.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Daniel Nebert, M.D. and Bernice T. Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 3/30/74
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $26,275

CONTRACT RESEARCH SUMMARY

Title: Effect of Non-Contraceptive Estrogens on the Occurrence of Breast Cancer

Principal Investigator: Robert W. Morgan, M.D.
Name/Address University of Toronto
Performing Toronto, Ontario, Canada
Organization:

Contract Number: NO1-CB-53969
Starting Date: June 30, 1975 Expiration Date: Dec. 29, 1976

Goal: To examine the risk of breast cancer, benign breast disease, and endometrial cancer in menopausal and postmenopausal women who had received estrogen therapy for other than contraceptive purposes.

Approach: A retrospective cohort study design will be used. The study group (Cohort A) will be composed of 1200 women (ages 40-55) in Toronto who between 1960 and 1970 were started on long-term estrogen therapy for menopausal complaints. All drug use and epidemiologic data will be gathered from hospital records, Ontario Cancer Registry, Ontario Cancer Foundation, private physician records, phone and home interviews. The incidence rates for breast cancer, benign breast disease and endometrial cancer will be compared with age-specific cancer incidence and mortality rates of the general population of Ontario (Cohort B). The incidence rates for the study population will also be compared with another 1200 matched control (Cohort C, unexposed to estrogens) group, drawn from friends of study group A. An estimation will be made of relative and attributable risk of developing benign or malignant breast disease or endometrial carcinoma and of dose-response relationships of estrogens to disease.

Progress: Questionnaires have been pretested. In two hospitals contacts were established and record search completed. We have established liaison with 17 private gynecologists. Two hundred and ninety-five exposed women have been identified from the above two sources. Letters seeking cooperation have been sent to 219 women from their physicians. Sixteen women have been interviewed and others are being contacted after receiving consent forms from them. Access to the Ontario Cancer Registry is now assured.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Elizabeth P. Anderson, Ph.D., Benjamin Hankey, Sc.D.
Program: Breast Cancer Epidemiology Site Visit Date: 2/17/76
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $74,300

CONTRACT RESEARCH SUMMARY

Title: Possible Correlations Between Morphology and Epidemiology of Breast Cancer

Principal Investigator: Bjorn Stenkvist, M.D.
Name/Address Department of Clinical Cytology
Performing University Hospital, Uppsala
Organization: S-750 14 Uppsala, Sweden

Contract Number: NO1-CB-53968
Starting Date: June 30, 1975 Expiration Date: June 29, 1977

Goal: To ascertain possible correlations between morphologic variables such as cytologic and histopathologic classifications and established epidemiological risk factors of breast cancer, such as menarche, age at first pregnancy, familial history, etc.

Approach: This study will be composed of two phases: Phase I will consist of a pilot study of histopathologic material from breast cancers from four counties in Sweden utilizing the computerized Central Swedish Cancer Registry. The tumors will be classified histologically according to WHO (Scarrf and Torloni 1968) and Ackerman's Classification (1970). Another aim of this project is to make a quantitative evaluation of a number of morphological characteristics by computer analysis of tumor cell populations utilizing a Leitz MPV II scanning photometer connected to a PDP-8 computer with 24k of core and relate these to the standard classification. Controls matched by age and place of living will be examined and interviewed in the same way as breast cancer patients. All epidemiologic data will be collected on each patient and computerized. Under Phase II statistical analysis of the data will be made with application of the newly established morphologic classifications to the epidemiologic variable.

Progress: Specimens have been collected from 180 breast cancer patients. These are presently classified according to the principles outlined above. 180 matched controls have just been invited for examination. Data are succesively transferred to magnetic tape via punch cards. These data will be analyzed with log linear statistical methods.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Bernice T. Radovich, Ph.D., Cecil Fox, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 1/17/75
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $118,929

CONTRACT RESEARCH SUMMARY

Title: Breast Cancer and Estriol Dynamics

Principal Investigator: Christopher Longcope, M.D.
Name/Address Worcester Foundation for
Performing Experimental Biology
Organization: Shrewsbury, Massachusetts 01545

Contract Number: NO1-CB-33902
Starting Date: June 1, 1973 Expiration Date: May 31, 1976

Goal: To determine the production and metabolic clearance of estriol in relation to high and low excretors in order to determine the physiological basis for repeated differences between high and low breast cancer-prone individuals.

Approach: In this research the investigator plans to apply proven techniques to determine the sources and blood production rates of estriol in (1) normal women with high and low urinary E_3/E_2+E_1 ratios; the women to range in age from 21 to 90 years of age and to be nulliparous as well as parous; and (2) women with breast cancer. The aim is to determine the pattern of precursor-estriol relationship and blood production rates in normal women who have high or low urinary $E_3/E_2/+E_1$ ratios, and in women with breast cancer and to correlate the dynamics as determined by measurements made from the free estriol pool in blood with those measurements made from estrogen metabolites in urine. These data will be used to delineate better the normal dynamics of estriol production and metabolism and the role of estriol in human breast cancer.

Progress: Studies have been carried out on 34 subjects with infusions of androstenedione, estrone, estradiol and estriol in the follicular and luteal phases of the cycle. There were no significant differences between the metabolic clearance rates, plasma concentrations and blood production rates of estriol in normal reproductive-aged women who have high as compared to low ratios. In both groups follicular phase concentrations and blood production rates were lower than those in the luteal phase. Sources of estriol and production rates of androstenedione, estrone and estradiol also did not differ significantly between the high and low ratio groups of normal women when measured in the respective follicular or luteal phases. In women who have had breast cancer estriol dynamics appeared similar to those in normal women.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 3/23/73
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY76 Funds: $42,450

CONTRACT RESEARCH SUMMARY

Title: Effects of Nucleic Acid Preparations on Biological Properties of Mammary Cancer

Principal Investigators: Janet S. Butel, Ph.D., Florence Farber, Ph.D.
Name/Address Baylor College of Medicine
Performing Organization: Houston, Texas 77025

Contract Number: NO1-CB-53904
Starting Date: 4/1/75 Expiration Date: 3/31/77

Goal: To determine the relationship between transfer of nucleic acid preparations into cells and possible changes in their growth potential.

Approach: In-depth study of gene transfer into somatic cells will be undertaken to: 1) Determine favorable conditions for the uptake of foreign DNA by cells, using various facilitators with purified DNA preparations; 2) Demonstrate biological activity by the heterologous DNA using specific markers: a) HGPRTase activity; b) induction of mouse mammary tumor virus (MTV); 3) Quantitate the intracellular fate of exogenous DNA in recipient cells; 4) Determine effect of exogenous DNA on growth properties of mouse mammary tumor cells.

Progress: Facilitators as polyornithine, $CaCl_2$, DEAE-dextran, spermine, polylysine polyarginine, and latex spheres have been tested for their ability to promote the uptake of exogenous DNA by mammalian cells. To date, polyornithine appears to enhance uptake to a greater extent than other facilitators tested. However, using herpes simplex virus DNA (100×10^6 mol. wt.) as an advantageous probe to monitor biological expression by the incorporated exogenous DNA, it appears that polyornithine does not promote biological activity nearly as effectively as $CaCl_2$. A series of mammary tumor cell lines derived from tumors which arose in mice spontaneously, i.e., after treatment by a hormone or a chemical carcinogen, were established in tissue culture. Two of the tumor lines have been cloned. Preliminary characterization of the growth properties of these tumor cell lines have been initiated. All of the cell lines, except one, exhibited an epithelioid morphology. Four of the 5 cell lines regrew when implanted into BALB/c mice. Such studies are preparatory to experiments designed to determine the effect of exogenous DNA on growth parameters of the cells. An RNA-dependent polymerase assay and a radioimmunoassay have been developed to detect mammary tumor virus and C-type oncornaviruses. Using the polymerase assay, studies are under way to determine the extent of MTV expression in the tumor cell lines and the possible induction of virus expression by dexamethasone. Conditions for the uptake of exogenous DNA by representative tumor cell lines have been examined.

Significance for Cancer Research (NCP 3 Approach 3)
Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Visit Date: 8/6/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $80,000

CONTRACT RESEARCH SUMMARY

Title: Role of Stroma in Neoplasia of the Mammary Gland

Principal Investigator: Peter O. Kohler, M.D.
Name/Address
Performing Organization: Baylor College of Medicine
Houston, Texas 77025

Contract Number: N01-CB-43907
Starting Date: 6/10/74 Expiration Date: 6/9/77

Goal: Determine the relationship between stromal and other mesenchymal cells on growth and differentiation of normal and malignant epithelial cells from the mouse mammary gland.

Approach: Develop and utilize mammary cell culture systems in which normal and malignant epithelial cells can be co-cultured in presence of mesenchymal cells either in direct contact or separated by a filter to permit passage of only cell products. Characterize cloned cell strains from mouse HAN and mammary tumor, as well as rat mammary tumor with regard to growth rates, hormone receptors, precursor incorporation into DNA and protein, and differentiation using light microscopy. Study the effect of stromal cells from various sources as embryonic mouse cells, defatted stroma from mammary fat pads, and WI-38 cells on the growth of the characterized cells listed above in co-culture or in separated filter and perfusion chamber culture. Study the specificity of various mammary fat depots in regulating growth of normal and pre-neoplastic epithelial cells.

Progress: Several strains of HAN cells from D_2 mice were isolated and cloned. Two uncloned and one cloned group of cells were transplanted into BALB/c subcutaneously and intrafat pad and all grew as sarcomatous cells. The HAN cells have been cultured with stromal cells on opposite sides of various supporting filters in a special perfusion chamber to determine the effect of stroma on growth and differentiation. New cloned strains are also being developed. Several cloned strains of DMBA-induced rat mammary tumor from Sprague-Dawley rats have been isolated. Two of these exhibit a growth response to 10^{-9}M estradiol in the medium. These rat mammary tumor cells as well as R3230AC are being tested in the culture chamber. A series of experiments have been set up to investigate the effect of normal cells on the growth of nodule cells *in vivo*. Single cell suspensions of nodule cells, normal cells, and a mixture of the two phenotypes have been implanted intrafat pad. The techniques for nodule cell dissociation, and successful cell transplantation at low concentrations have been accomplished. Titration of nodule cells and the mixing experiments are in progress. A series of experiments have demonstrated that mammary white fat provides an environment necessary for nodule growth *in vivo*. The parametrial fat depot and white fat subcutaneously will not support nodule growth. Follow-up experiments are in progress. A long term experiment concerning the effects of chemical carcinogens on the ability of mammary stroma to influence the growth and tumor potential of nodule tissue has been completed. Neither MCA nor DMBA alter the ability of mammary stroma to support the growth and tumor potential of a low tumor line D_1 or the high tumor line D_2.

Significance for Cancer Research (NCP Objective 4 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., Richard A. Knazek, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/15/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: Approx. $95,000

CONTRACT RESEARCH SUMMARY

Title: Role of Prolactin in Breast Cancer

Principal Investigator: Olof H. Pearson, M.D.
Name/Address Case Western Reserve University
Performing Organization: Cleveland, Ohio 44106

Contract Number: N01-CB-23859
Starting Date: 6/26/72 Expiration Date: 6/25/77

Goal: To find specific receptor sites for prolactin, estrogens and progesterone which might predict hormone responsiveness of individual breast tumors.

Approach: Human and animal mammary tumor tissue for the following studies: 1) Detect and quantify prolactin receptors in membrane fractions of mammary tumors in animals and humans; 2) Measure estrogen and progesterone receptors in mammary tumors; 3) Correlate receptor findings in tumors with response to treatment with antiprolactin drug (Lergotrile), with antiestrogen drug (Tamoxifen) and ablation (hypophysectomy).

Progress: Prolactin (PRL) receptors are abundantly present in DMBA-induced rat mammary cancers and are present in low titers in about 30% of human mammary cancers. A significant correlation exists between the presence of PRL receptors and hormone dependence in DMBA tumors. Hormonal factors influence the number of PRL binding sites in rat mammary cancers and in liver, and PRL appears to play a major role in regulating its own receptor sites. Anti-PRL treatment (Lergotrile) of 12 patients with metastatic breast cancer produced arrest of the disease in 4 patients and was not as effective as other endocrine modalities. Estrogen receptors (RE) in human mammary cancers are predictive of the response of the patient to hypophysectomy. ER in DMBA tumors and uterus are not influenced by hormonal factors but depend on the integrity of the tissue. Antiestrogen treatment (Tamoxifen) induces striking objective remissions in both pre- and postmenopausal women and is being compared to hypophysectomy. Progesterone receptors are in 60% of ER positive human breast cancers and absent in ER negative tumors. Clinical correlations are under way.

Significance of Cancer Research (NCP Objective 5 Approach 6)

Project Officer: D. Jane Taylor, Ph.D., Harold Hoffman, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/18/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: Approx. $89,972 Terminal

CONTRACT RESEARCH SUMMARY

Title: Identification of Cell Types in Cultures of Normal and Neoplastic Breast Tissues

Principal Investigator: Samuel Abraham, Ph.D.
Name/Address Children's Hospital Medical Center, Bruce Lyon
Performing Organization: Memorial Research Laboratory, Oakland, Calif. 94609

Contract Number: NO1-CB-33906
Starting Date: 6/27/73 Expiration Date: 6/26/76

Goal: To identify cell markers in normal and neoplastic breast cultures of mouse, dog and human origin.

Approach: Antisera to be prepared from: intact cells, cell membranes, microsomes, specific enzymes or cell products. Normal epithelial cells from mouse mammary gland to be separated with methods developed by Abraham et al. Adipose and fibroblast cells to be obtained from cleared fat pad. Epithelial cell identification will be studied by cell membrane antigens. Specific antibodies produced for each cell type to be tagged with either fluorescent dye or peroxidase. Alternative markers for identification of cell types, such as hormone binding, specific products or enzymes, to be explored. Unique isoenzymes are a possibility for distinguishing normal and neoplastic cells.

Progress: Antibodies were prepared in rabbits against cultured mouse mammary epithelial cells (MMEC) and in guinea pigs against cultured mouse mammary fibrocytes (MMFC). These antibodies will distinguish between MMEC and MMFC with the use of a two stage fluorescent staining procedure. MMEC, after enzymtic dissociation from tissue, were found capable in vitro of regenerating those cell surface components responsible for antigenic properties. Enzymatically removed material contains antigenically intact components that bind specifically to an immunoadsorbent of anti-MMEC and Sepharose 4B. Evidence to support the view that these antigens are not merely produced in vitro, but are present in intact tissue has been obtained. Anti-MMEC prepared from BALB/c cells are not strain specific, it reacts with several other mouse strains (C57/Bl, A, GR/A, C_3H). Progress has been made towards obtaining antibodies specifically directed against tumor cells (MMTC). MMEC and MMTC seem to share many common antigens. Analysis of cell surface components obtained from the two cell types reveals that different substances useful for antibody production could be obtained. Mild enzymatic treatment of intact cells will solubilize intact antigenic materials originally present on cell surfaces. Analyses of these fractions reveal that tumor cells lack specific components present on normal cells. Since these materials contain fucose and amino acids capable of iodination by the lactoperoxidase method, we conclude they are present on the cell surface and are probably glycoprotein in nature.

Significance for Cancer Research (NCP Objective 3 Approach 1)
Project Officer: D. Jane Taylor, Ph.D., Harold Hoffman, Ph.D.
Program: Cancer Experimental Biology Site Visit Date: 3/13/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: None; Terminal

CONTRACT RESEARCH SUMMARY

Title: Pathological History of the Mammary Gland in Pseudohermaphroditic Rats and Mice

Principal Investigators: Susumu Ohno, D.V.M., Ph.D., D.Sc.
Name/Address James Kan, Ph.D.
Performing Organization: City of Hope Medical Center
Duarte, California 91010

Contract Number: NO1-CB-33907
Starting Date: 6/27/73 Expiration Date: 6/26/77

Goal: To study the underlying mechanism of the inhibitory action of testosterone on mammary gland development by the use of the X-linked Tfm (testicular feminization) mutation of the mouse.

Approach: Study of the role of 7,12-dimethylbenzanthracene (DMBA) on hypothalamic function as to the cause of mammary tumor development by: 1) Determining whether or not excessive luteinization, observed at each estrus cycle of DMBA-treated female mice, is due to the excessive release of pituitary LH and prolactin. 2) Elucidating the reason way DMBA, administered through a stomach tube, specifically induces mammary carcinomas in female rats (prolactin effect?), while inducing, instead, ovarian granulosa cell tumors in female mice (LH-effect?). 3) Using tritiated DMBA, determine: whether it concentrates in the hypothalamic region of the brain; and whether its metabolites have a high binding affinity to androgen or estrogen receptor proteins in this region.

Progress: 1) Using natural mosaicism to androgen response in Tfm/+ heterozygous females, we have shown that the suppressive action of androgen of lactating mammary glands is not direct but is due to the negative feedback inhibition of pituitary prolactin. 2) Two 5 mg doses of DMBA, administered through a stomach tube on successive days, restored estrus cycles to, and abolished masculine behavior from neonatally masculinized female mice in "persistent estrus." The positive feedback response to a rising estrogen level restored by DMBA to the masculinized hypothalamus was excessive, for overluteinization, occurring at each estrus cycle, quickly exhausted the ovarian stock of oocytes and caused the development of granulosa cell tumors. 3) 0.17 mg DMBA neonatally administered, caused the precocious menopause in female mice (120 days old) which was associated with the development of generalized lymphomas, mammary adenocanthomas and ovarian granulosa cell tumors.

Significance for Cancer Research (NCP Objective 1 Approach 4)

Project Officer: D. Jane Taylor, Ph.D., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/10/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: Approx. $90,883

CONTRACT RESEARCH SUMMARY

Title: Protein Nucleic Acid Interactions in Transcription of Normal and Cancer Cells

Principal Investigator: Kenneth S. McCarty, Ph.D.
Name/Address Duke University
Performing Organization: Durham, North Carolina 27710

Contract Number: NO1-CB-23857
Starting Date: 6/29/72 Expiration Date: 12/28/76

Goal: To elucidate the role of proteins of the mammary cell nucleus in the expression and regulation of genetic information of normal and maligant cells.
Approach: Improving techniques for the isolation of chromatin and the extraction of acidic nuclear proteins. DNA-protein rebinding techniques to be used with isolated nuclear protein to determine the comparative affinities for phosphorylated and dephosphorylated acidic chromosomal proteins. Techniques of chromatin reconstruction and transcription to be used to determine effect of hormones. Chromatin interactions with phosphorylated and dephosphorylated proteins. Examine RNA synthesized as a result of prolactin exposure to intact cells and isolated nuclei. Determine inductive capacity of hormones on mammary gland polyamine biosynthesis. Fischer/344 rat mammary tissue, normal and neoplastic (13762 adenocarcinoma) is being utilized in addition to studies of C3H mouse mammary tissue.
Progress: Developed techniques for preparation of nuclei, histones and chromosomal proteins for Fischer/344 rat mammary gland. Techniques were modified for mammary gland tissue compared to other tissues as spleen, brain, liver, etc. Homogenization with hexylene glycol was essential for mammary gland fractionation and isolation of pure nuclei. NP 40 was required to strip the rough endoplasmic reticulum and remove nuclear membrane components assuring no contamination of acidic chromosomal proteins. Nuclei were fractionated with a Bronson sonifier into 3 components: soluble fraction chromatin supernatant, membrane-interphase, nucleolar-pellet. The chromatin was dissociated utilizing gradients of lithium chloride, 5 M urea, TOC, into histones and acidic chromosomal proteins. Cetyltrimethylammonium bromide (CTAB) was used to remove DNA and further separate RNA from DNA. Since cell suspension cultures fail to respond to hormones, an apparatus was developed which will become commercially available for mass culture of surface contacted mammary gland cells. On the basis of specific hydrophoresis interactions F2a2 histones have been fractionated on Triton gel electrophoresis to give multiple components which exhibit differing degrees of phosphorylation as a result of hormone induction. Several papers were published in this contract period by McCarty and colleagues.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 7/25/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $45,700 Terminal

CONTRACT RESEARCH SUMMARY

Title: The Response of the Embryonic Mammary Gland to Androgenic Hormones

Principal Investigator: Klaus Kratochwil, Ph.D.
Name/Address Institute for Molecular Biology
Performing Organization: Salzburg, AUSTRIA

Contract Number: NO1-CB-33883
Starting Date: 6/29/73 Expiration Date: 3/28/77

Goal: To study the acquisition of hormone responsiveness during development of the mammary gland. Analysis of the androgen response with special emphasis on the role of tissue interaction.

Approach: Determine whether sensitivity of mammary tissue to androgen exists during the entire developmental period or is it acquired at a specific time. Mammary gland buds of various developmental stages are exposed to testosterone in vitro. Hormone binding of mammary tissue to be studied in its correlation with acquisition of hormone responsiveness. Concentrate in the year ahead on: finding the basis for the transient androgen responsiveness of the embryonic mammary gland rudiment; analyze processes by which the mesenchyme (the target tissue for testosterone) affects epithelial development, with special attention to tissue and cell interaction; and complete the morphological study of the response itself and obtain data on the fate and of role of tissue interface material, e.g., basal lamina, in the androgen response.

Progress: Established that the androgen-sensitive phase in mammary gland development lasts only from day 13 to day 15 of gestation. Further established that the morphological response to testosterone of explanted mammary gland rudiments is identical to that found in male fetuses. This made it possible to work out a sequence of events on explants exposed to testosterone for precisely known periods. The outstanding feature is the disappearance of the basal lamina before the onset of necrosis. A difficult technical problem in the analysis of tissue interaction, the enzymatic separation of mammary epithelium from all adhering mesenchymal cells have been overcome. This has made it possible to identify the mesenchyme as the primary target tissue for testosterone in Tfm/y - wild type tissue combinations. This result represents a major step forward in the analysis of the androgen-induced tissue destruction.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 9/20/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $50,000

CONTRACT RESEARCH SUMMARY

Title: Animal Mammary Tumors and Humam Cell Bank Facility

Principal Investigator: Arthur E. Bogden, Ph.D.
Name/Address Mason Research Institute
Performing Organization: Worcester, Massachusetts 01608

Contract Number: NO1-CB-31146
Starting Date: 6/28/63 Expiration Date: 6/27/77

Goal: To maintain a bank of biologically characterized and monitored (a) in vivo transplantable animal mammary and endocrine related tumors, and (b) human and animal breast tumor cell lines established in culture, to be used by the BCTF and other selected investigators.

Approach: Freeze-preservation of human breast cell lines, normal and abnormal, and mammary and other endocrine related tumors of animal origin: (1) submittal of tumors and cell lines by qualified investigators able to furnish pertinent background information; (2) cell lines tested for viability, plating efficiency, freedom from contamination and tumorigenicity by implanting in nude athymic mice; (3) biological characterization of transplantable tissues, both pre- and post-freeze preservation, by determination of growth curves, specific effects on host-organs, host survival, serum hormone levels, histology, karyology, response to ablative procedures; (4) characterized tissue and cell lines preserved in Linde liquid nitrogen freezers according to recognized, proven procedures.

Progress: Tumors (122) have been shipped to 62 investigators reflecting 123% increase over the past year. Current inventory of the Tumor Bank includes 118 different tumors or tumor sublines. Five new tumors have been received recently for characterization and freezing. Twenty-four tumors have been serially transplanted for characterization studies and 13 for replenishing the Tumor Bank inventory. The Cell Bank contains 7 human cell lines, 5 of neoplastic origin and 2 of normal origin, and one rat mammary adenocarcinoma. All cell lines have been tested and are free of bacterial, fungal, and mycoplasma contamination. Morphological and growth characteristics conform to those of the original cultures. All cell lines have been tested for growth in soft agar and tumorigenicity studies in nude mice are in progress. A total of 36 shipments of viable cultures or frozen ampules were made to 22 different laboratories in the past year.

Significance for Cancer Research (NCP Objective 3,4,5 Approach_____)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/4/71
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: Approx. $171,906

CONTRACT RESEARCH SUMMARY

Title: Studies of the Role of Prostaglandins in Mammary Gland Neoplasia

Principal Investigator: Dwight R. Robinson, M.D.
Name/Address Massachusetts General Hospital
Performing Organization: Boston, Massachusetts 02114

Contract Number: NO1-CB-33918
Starting Date: 6/30/73 Expiration Date: 6/29/76

Goal: To determine whether there are differences in prostaglandin (PG) synthesis by normal and neoplastic human breast tissue. To investigate the role of prostaglandins in growth and differentiation.

Approach: Production of PGE and PGF by benign and neoplastic human breast tissue will be measured. Surgical specimens are maintained in short term organ culture and the media analyzed for PGE, PGF and tissue and media for cyclic AMP by radioimmunoassays (method of Levine for PGs). Comparisons will be made between PG and cAMP between normal breast tissues, cystic disease, fibroadenomas and carcinomas. Correlations between these data and the clinical properties of the malignancies will be attempted. In a few breast cancer patients, PG analyses of regional venous blood draining the tumor and peripheral venous blood will be compared. The production of PG by human mammary cell lines (provided by Dr. E.M. Jensen) will be measured and the effect of indomethacin, and inhibitor of PG synthesis, on the growth of these cells in culture will be examined. PG production by normal and rheumatoid synovial and skin fibroblast cell strains will be compared to mammary tumor cell lines. Explant cultures of synovia and skin will also be compared to explants of mammary tissues. Chromatographic techniques have been set up to aid in characterization of PGs. The effects of indomethacin, colochicine and other drugs on PG and cAMP production will be investigated.

Progress: Experiments have been successfully completed with organ cultures from 22 patients with breast cancer, 8 fibrocystic disease, 3 fibroadenomas, and 3 normals. The mean PGE levels from 3-day incubations (days 3-6) in ng/ml/mg wt weight tissue are: cancer 3.84, normal and fibrocystic disease 0.18, fibroadenoma 5.24. Thus cancer tissue gave ∿20-fold greater PGE levels than normal and fibrocystic disease. Duplicate plates were incubated in the presence of indomethacin (5 µg/ml) for all tissues and gave >90% inhibition of PG production in nearly all cases. Over half the tissue and replicate plates incubated with colchicine (0.1 µg/ml) which led to stimulation of PG synthesis in a majority of tissues, >10-fold in some cases, suggesting that microtubules may regulate PG biosynthesis. Approximately half these tissues had cultures carried out after dispersion following collagenase digestion and in these preparations the differences between cancer and benign tissues are reduced compared to the explant technique. Careful histologic examination of the tissues is being carried with the aid of pathologist, in order to relate the detailed morphology to PG production in culture.

Significance for Cancer Research (NCP Objective 4 Approach 1)
Project Officer: D. Jane Taylor, Ph.D., William Kidwell, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 5/4/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: None; Terminal

CONTRACT RESEARCH SUMMARY

Title: Role of 3', 5'-cAMP and Related Enzyme Systems in Mammary Neoplasia

Principal Investigator: David F. Scott, Ph.D.
Name/Address Medical College of Georgia
Performing Organization: Augusta, Georgia 30902

Contract Number: NO1-CB-33909
Starting Date: 6/30/73 Expiration Date: 4/29/76

Goal: To determine the involvement of 3', 5'-adenosine monophosphate (cAMP) in the proliferation of normal and neoplastic mammary tissues.

Approach: The different types of cells in normal and developing mammary gland and neoplastic cells of mammary tumors will be isolated. The hormonal regulation of cAMP levels will be studied using mammary tissue explants and isolated cells. The activities of adenylate cyclase and phosphodiesterase will be measured in homogenates of whole glands, isolated cells, mammary gland explants and cell cultures. A comparison of growth rates and cAMP levels in normal and neoplastic cells and tissues will be made. The main objective is to understand the mechanism(s) by which cAMP levels are controlled in mammary tissues.

Progress: Epinephrine (EP) and prostaglandin E_1 (PGE) treatments elevated cAMP levels in mammary gland explants from virgin, pregnant and lactating mice whereas prolactin (PRL) was effective only on those from virgin or lactating mice. Freshly prepared adipose and epithelial cells as well as primary cultures of fibroblasts and epithelial cells derived from mammary glands of virgin, pregnant, and lactating mice were incubated with each agent. Each responded to EP and PGE, but only adipose cells responded to PRL. When tumor explants were studied, 20 of 21 responded to PGE, and 4 of 21 responded to PRL. When expressed as a function of tissue weight, cAMP levels in tumors are higher than in normal mammary tissues. However, this difference vanishes when cAMP levels are expressed on the basis of tissue protein or DNA. No relationship between cAMP levels and tumor growth rate is apparent. Mammary cAMP levels decrease in midpregnancy, increase in late pregnancy and remain elevated during lactation. Addition of serum and insulin to explants incubated 48 hours in McCoy's medium resulted in a biphasic increase of cAMP level prior to DNA synthesis. Seven cell lines were established and karyotyped; 2 mammary tissue and 5 mammary tumor. Six lines were tumorigenic.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., William Kidwell, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 4/20/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: None; Terminal

CONTRACT RESEARCH SUMMARY

Title: Identification of Mammary Tissue

Principal Investigator: Melvyn S. Soloff, Ph.D.
Name/Address Medical College of Ohio
Performing Organization: Toledo, Ohio 43614

Contract Number: NO1-CB-63983
Starting Date: Expiration Date:

Goal: To isolate, identify, characterize and quantitate myoepithelial cells in rat mammary tissue and tumors of rat and human origin.

Approach: Separate myoepithelial cells by density gradient centrifugation and sedimentation in bovine serum albumin gradients. Obtain specific antisera against these cells. Use this antisera for quantitation studies. Use (^{3}H) oxytocin as a marker for myoepithelial cells. Study the potential of alkaline phosphatases and ATPase as markers. Myoepithelial cells will be isolated from mammary glands of virgin, pregnant and postpartum rats, tumors induced with DMBA and human tumors obtained from surgery.

Progress: New Contract

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer:
Program: Breast Cancer Experimental Biology Site Visit Date: 2/13/76
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: Approx. $130,691

CONTRACT RESEARCH SUMMARY

Title: Biochemical Means by Which Effector Molecules Bind to Mammary Cell Surfaces

Principal Investigator: Arnold D. Rubin, M.D.
Name/Address Mount Sinai School of Medicine
Performing Organization: New York, New York 10029

Contract Number: NO1-CB-33912
Starting Date: 6/28/73 Expiration Date: 3/27/76

Goal: Analysis of the effects of lymphocyte effector molecule mammary cell interaction.

Approach: To purify the reversible inhibitor of lymphoid cell growth from SKBR-3 human breast cancer cell line, and to delineate the mechanism which produces the effect and to examine its potential to prevent antigen stimulated growth. Other breast cancer cell lines will be examined for inhibitor. Analyze lymphocyte growth in response to antigens extracted from the cell lines and from extracts of tumor tissue.

Progress: Further investigations employed the HBT-3, BT-20 (insulin-dependent) and SKBR-3 mammary cancer derived lines. Inhibitor obtained from SKBR-3 cells and employed at culture concentrations of 1 mg/ml significantly inhibits the PHA-response of normal lymphocytes, even when lymphocyte exposure to the inhibitor is minimal (2-6 hours). This suggests a block in early events associated with lymphocyte blastogenesis. Alternatively, medium obtained from PHA stimulated lymphocyte cultures and subsequently used to culture SKBR-3 cells enhanced the macromolecular RNA metabolism of the breast line cells. Since the effect was not due to PHA, one may assume that lymphoid humoral factors (possibly blastogenic factor) could be responsible for the observed effects. Quantitative studies of galactosyl transferase activity (expressed as pico moles galactose-^{3}H/mg protein/10 minutes) on HBT-3 cells demonstrated an 8-fold increase in alpha lactalbumin activity following sequestial addition of insulin, hydrocortisone and prolactin. Similar studies on the BT-20 (insulin-dependent) cells produced no such increases. These quantitative studies will be extended to the SKBR-3 line. Studies will be extended to check on inhibitor production by other established mammary cancer cell lines and to determine if humoral agents produced by growing lymphocytes affect other cell lines besides SKBR-3.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/30/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: None; Terminal

CONTRACT RESEARCH SUMMARY

Title: Biochemical Means by Which Effector Molecules Bind to Mammary Cell Surfaces

Principal Investigators: Kermit L. Carraway, Ph.D., Kurt E. Ebner, Ph.D.
Name/Address Oklahoma State University, Stillwater,
Performing Organization: Oklahoma 74074, and University of Kansas Medical Center, Kansas City, Kansas 66103

Contract Number: NO1-CB-33910
Starting Date: 6/27/73 Expiration Date: 6/26/76

Goal: To study composition and biochemical properties of normal and neoplastic mammary cell plasma membranes.

Approach: Single cell suspension from normal lactating mammary gland and tumors of rats will be prepared by enzymatic procedures and fractionated in various gradients or prolactin affinity columns. Cell surface repair will be evaluated by incorporation of radioactive fucose, glucosamine and leucine. Surface structures of normal and tumor membranes will be evaluated by gel electrophoresis, glycoprotein patterns, lectin binding, susceptibility to proteolysis, iodination with lactoperoxidase. The purity of isolated cell membranes will be assessed by biochemical and microscopic methods. Quantitative binding of prolactin to cell membranes will be determined.

Progress: Plasma membrane enriched fractions were prepared from lactating mammary tissue and R3230AC adenocarcinoma by fractionation of a tissue microsomal fraction. Plasma membranes were prepared from the 13762A ascites mammary adenocarcinoma. Isolated R3230 cell suspensions were obtained by mechanical disruption and mild enzymatic treatment. Membranes isolated from these cells compare well to those from tumor tissue in terms of protein and glycoprotein distributions. Presence of galactosyltransferase activity in the plasma membrane preparations indicated Golgi contamination. Will attempt to eliminate the contaminant by subfractionation of the membranes. Concanavalin A inactivates at least two forms of mammary 5'-nucleotidase by a cooperative process involving direct interaction with the enzyme. Mg^{++}-ATPase is activated by Con A. Prolactin receptor from rabbit mammary particles was solubilized and its purification by column chromatographic methods tested. Isolation of prolactin receptor from rat tissue was complicated by its instability.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: D. Jane Taylor, Ph.D., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/8/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: None; Terminal (at investigators' request)

CONTRACT RESEARCH SUMMARY

Title: Glycoproteins of the Mammary Cell Surface

Principal Investigator: Kermit L. Carraway, Ph.D.
Name/Address Oklahoma State University
Performing Organization: Stillwater, Oklahoma 74074

Contract Number:
Starting Date: Expiration Date:

Goal: To characterize the glycoproteins on the tumor cell surface and determine their release from the cells.

Approach: Cell cultures of normal rat mid-pregnant and lactating tissue as well as R3230AC and 13762 rat mammary adenocarcinomas will be studied. Isolate glycoproteins from the cell membranes and purify these by gel filtration in SDS. Prepare antibodies against these purified glycoproteins. Analyze the 5'-nucleotidase enzymes on whole cells, homogenates and isolated membranes. Isolate and purify the 5'-nucleotidase and prepare antisera against it. Study the turnover of membrane glycoproteins of the mammary tumor cells.

Progress: New contract

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer:
Program: Breast Cancer Experimental Biology Site Visit Date: 2/3/76
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: Approx. $132,753

CONTRACT RESEARCH SUMMARY

Title: Glycoproteins of the Mammary Cell Surface

Principal Investigator: Eugene A. Davidson, Ph.D.
Name/Address Pennsylvania State University
Performing Organization: Hershey, Pennsylvania 17033

Contact Number: NO1-CB-63984
Starting Date: Expiration Date:

Goal: To isolate and characterize the cell surface glycoproteins and other complex carbohydrates produced by rat and human mammary carcinoma cells.

Approach: Apply techniques for surface labelling of the protein and carbohydrate moieties of the glycoproteins on the cell plasma membrane. Assess the labelled macromolecules in terms of molecular size and localization within the membrane. Apply the best method for isolation and purification of plasma membranes. Characterize the purified plasma membrane for their chemical architecture, biological, immunological properties, and enzyme markers. Determine the relationship of cell surface glycoprotein synthesis and release to the cell cycle, to cell density and metastasizing potential. Human mammary cell lines obtained for the BCTF Cell Bank will be used for the studies.

Progress: New Contract

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer:
Program: Breast Cancer Experimental Biology Site Visit Date: 2/17/76
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: Approx. $147,058

CONTRACT RESEARCH SUMMARY

Title: Cultivation of Normal and Malignant Human Mammary Tissue

Principal Investigator: Edwin V. Gaffney, Ph.D.
Name/Address Pennsylvania State University
Performing Organization: University Park, Pennsylvania 16802

Contract Number: NO1-CB-33898
Starting Date: 6/29/73 Expiration Date: 3/27/77

Goal: Isolate and maintain pure cultures of human epithelial cells derived from normal and malignant breast tissues as well as from milk. To determine factors which enhance or depress proliferation of malignant cells in vitro. To establish if certain lactogenic hormones will predispose normal breast cells to malignant alterations.

Approach: In vitro culture study of normal and malignant cells. Single cell suspensions of normal and malignant breast tissue prepared with collagenase and trypsin. Cells separated by sedimentation at unit gravity in bovine serum albumin gradients. Use of polyacrylamide gel to isolate epithelial cell types without fibroblast overgrowth. Isolate normal or pre-malignant epithelial cells from aspirated human milk samples. Hormone effects on malignant and normal cells.

Progress: In vitro cultures have been established from tissues obtained from specimens at autopsy, reduction mammoplasty and lactating human breasts. Pure epithelial cultures were selected by seeding suspensions of polyacrylamide gels followed by transfer to plastic or glass surfaces. All cultures subjected to lactogenic hormones had increased DNA synthesis and phosphoproteins. Cell cultures derived from tissues of patients of various age groups demonstrated differences in formation of tumor antigen following SV40 infection in presence of prolactin and/or estradiol. Low concentrations of spermadine enhances whereas high concentrations inhibits DNA synthesis. Hormones are required in the media for cancer cells to replicate in vitro. Cell cultures always establish from tumors which metastasize but rarely establish from samples of small primary tumors. Two epithelial and two fibroblast-like cell lines were initiated from human milk samples obtained from 250 nursing mothers. Initial chromosomal mode studies were done on two lines and one cell line was inoculated into nude-athymic mice to determine tumorigenicity.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: D. Jane Taylor, Ph.D., Katherine Sanford, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/27/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $66,000

CONTRACT RESEARCH SUMMARY

Title: Isolation of Prolactin Cells from Human and Rat Adenohypophysis

Principal Investigator: Wesley C. Hymer, Ph.D.
Name/Address The Pennsylvania State University
Performing Organization: University Park, Pennsylvania 16802

Contract Number: NO1-CB-23863
Starting Date: 6/28/72 Expiration Date: 3/[illegible]6/77

Goal: To isolate mammotrophs from human and rat adenohypophysis, to cultivate them and study their effects on mammary tissue, normal and neoplastic.

Approach: The following material will be used: 1) Human adenohypophysis from surgical material. 2) Rat adenohypophysis from virgin, lactating, estrogen-treated and mammary ascites tumor-bearing females. The experiments will include: 1) Cell dissociation. 2) Cell separation by sedimentation at 1G in BSA gradient chamber (NIH model). 3) Culture of separated cells alone and in co-culture with normal and malignant mammary cells. 4) Prolactin radio-immunoassay.

Progress: Rat co-culture experiments showed: 1) In 39 experiments 34 (88%) showed elevated levels of prolactin released by pituitary cells in co-culture with mammary cells versus that from an equivalent number of pituitary cells alone. Twenty-two of the 34 experiments were statistically significant ($p<0.05$) 2) Pituitary cells of lactating rats respond better than those from untreated ones. 3) Separated mammary fibroblasts or epithelial cells gave a good response but cells from parotid, kidney, pineal, or mammary fat did not. 4) The 13762 rat mammary ascites tumor is a good model to study this response in that prolactin secretion from the pituitary of the host animal is suppressed. The human co-culture experiments showed: 1) Of 21 experiments, 16 (76%) showed elevated prolactin release when co-cultured with human mammary cells and 56% of these experiments were statistically significant. 2) Normal cells from reduction mammoplasty or cells from malignant tissue gave a consistent response whereas established cell lines as ALAB showed fewer responses. The results may be important in defining the hypothalamic pituitary-mammary gland axis. Some samples of human pituitary cells grew in aggregates, e.g., in one case the prolactin yield was approximately 0.5 µg prolactin/day/75,000 cells seeded during 44 days. About 25% of the mammotroph population from human breast cancer patients are hypertrophied. Since May 1974, 21 human pituitary glands were obtained: 11 from patients with breast cancer and 10 from assorted disorders, e.g., galactorrhea, acromegaly, adenomas, etc.

Significance for Cancer Research (NCP Objective 3 Approach 3)
Project Officer: D. Jane Taylor, Ph.D., Harold Hoffman, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/3/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $77,000

CONTRACT RESEARCH SUMMARY

Title: Studies on Basic Biological Events in the Pathogenesis of Mammary Cancer

Principal Investigator: Thomas L. Dao, M.D.
Name/Address Roswell Park Memorial Institute
Performing Organization: 666 Elm Street
Buffalo, New York 14263

Contract Number: N01-CB-23865
Starting Date: 6/15/72 Expiration Date: 6/14/77

Goal: Determine the role of hormones and other host factors in the pathogenesis of mammary dysplasia and neoplasia.

Approach: 1) Continue characterization of hyperplastic alveolar nodules (HAN) of rat mammary tissue by: cell kinetics consisting of cell proliferation rate in vivo, DNA synthesis, and cell cycle; karyotyping of chromosomes and their banding patterns. 2) Investigate the fate of HAN by: transplantation of individual HAN into subscapular fat pad of isologous recipient host and observe whether neoplastic transformation occurs; transplanting the whole mammary gland (with HAN) into the interscapular fat pad and following HAN growth; and studying the incidence of HAN in aging rats and determining their natural history. 3) Determine the malignant potential of HAN in vitro by exposure to low levels of DMBA and transplanting in vivo; also in vivo experiments with whole mammary gland (with HAN) transplanted into isologous host and host treatment of DMBA (low level). 4) Study the effect of DMBA metabolites on the induction of HAN in vivo and in vitro.

Progress: Have successfully cultivated normal, neoplastic and HAN mammary tissue from Sprague Dawley rats for long periods. Developed a technique for identification of HAN in vivo so they can be removed for biochemical and transplantation studies. Hormone responsiveness of the three kinds of tissue have been compared biochemically using insulin, estradiol, progesterone and prolactin; the data suggest that HAN contain cell populations which are not in proliferation as compared to those of mammary gland or the tumor. DMBA given intravenously to 'old' rats develops numerous HAN but no mammary tumors, while the same carcinogen given by local application induces mammary tumors but no HAN. The data do not support the concept that HAN is an intermediate step in the tumorigenesis of the mammary gland in the rat. DMBA-induced mammary tumors have been demonstrated to be of ductal origin. HAN transplanted into the interscapular fat pad develop either lobuloalveolar or ductal growth but not neoplasia. Biochemical characterization of HAN shows a lack of hormone dependence. One paper was published in 1974, two in 1975.

Significance for Cancer Research (NCP Objective 3 Approach 1)
Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Visit Date: 1/20/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: Approx. $80,000 Terminal

CONTRACT RESEARCH SUMMARY

Title: Studies on the Prevention of Metastasis in Mammary Cancer

Principal Investigator: Untae Kim, M.D.
Name/Address Roswell Park Memorial Institute
Performing Organization: Buffalo, New York 14263

Contract Number: NO1-CB-23864
Starting Date: 6/15/72 Expiration Date: 8/14/76

Goal: Determine the mechanism of mammary tumor metastasis in relation to antigen-shedding property of tumor cells and host immune response to free antigens, and to develop clinically applicable procedures for prevention of metastasis.

Approach: Utilizing established metastasizing (M) and non-metastasizing (NM) mammary tumors in W/Fu rats, the following lines of research are being pursued: 1) Biochemical analyses of tumor cells to learn the mechanism of antigen-shedding with respect to glycoprotein and glycolipid metabolism, in isolated plasma membranes, and the role of cyclic nucleotides and lysosomal enzymes. 2) Analysis of host immune responses to antigenic and nonantigenic, or antigen bound and antigen-shedding tumor cells in relation to in vitro migration pattern of peripheral lymphocytes, spleen cells and thymocytes from tumor hosts, and localization of free or bound antibodies in sera and on the surface of tumor cells and lymphocytes, in order to learn the immune escape mechanism of spontaneously M tumor cells. 3) Establishment of biochemical guidelines for simple vs. radical mastectomy in breast cancer patients. 4) Development of experimental therapeutic measures for preventing metastasis. 5) Immunology of prolactin receptors in various experimental mammary tumors in collaboration with Dr. Dandliker.

Progress: 1) Pathogenesis of spontaneously M mammary tumors has been established. 2) Surface antigen-shedding property has been shown to be responsible for loss of immunogenicity and acquisition of M capacity by the tumor cells. 3) Inverse relationship between M capacity and levels of plasma membrane marker enzymes has been established. 4) Direct correlation between endogenous cAMP and M capacity or antigen-shedding property, and inverse relationship with cAMP phosphodiesterase activity. 5) Spleen, thymus and peripheral lymphocytes from M and NM tumor hosts have different migration patterns in vitro, with or without specific tumor antigens. 6) M or antigen-shedding cells have greatly elevated glycosyltransferase activity in the plasma membranes. 7) Biochemical and immunological characteristics of the self-curing, spontaneously M tumor DMBA-4 have been analyzed.

Significance for Cancer Research (NCP Objective 4 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 1/20/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: Extended without funds.

CONTRACT RESEARCH SUMMARY

Title: Mammary Gland Responsiveness to Multiple Hormones

Co-Principal Investigators: W.B. Dandliker, Ph.D. and
Name/Address S.A. Levison, Ph.D.
Performing Organization: Scripps Clinic and Research Foundation
La Jolla, California 92037

Contract Number: NO1-CB-43905
Starting Date: 6/1/74 Expiration Date: 2/29/77

Goal: Determine mechanism(s) underlying the ability of the mammary gland to respond to several steroid and peptide hormones. Investigate possible competitive, cooperative or sequential effects in the binding of hormones to their specific cellular receptors of mammary tissue.

Approach: Fluorescent tagging of hormones, e.g., prolactin, growth hormone, insulin, estradiol, testosterone, corticosteroids, and purification of the fluorescent derivatives by chromatography. Equilibrium and kinetics of hormone binding to specific receptors is then monitored by changes in fluorescence polarization of the labeled hormones under a variety of conditions. Microsomal and cytosol receptors prepared from mammary tissues of pregnant rabbits and rat mammary tumor cell lines. Compare above with binding of labeled hormones by living cells as measured by fluorimetric techniques.

Progress: Fluorescence polarization measurements of the specific binding of prolactin and estradiol to isolated receptors and to whole cells are being pursued on both rat mammary ascites tumor 13762 and rabbit mammary tissue. Scatchard plots indicate both high affinity ($K \sim 10^9$) and low affinity ($K \sim 10^8$-10^7) sites for both hormones. The kinetic dissociation constant of the high affinity sites remaining bound after charcoal adsorption has been estimated to be $\sim 10^{-3} sec^{-1}$. Inhibition of the binding of fluorescent estradiol to its receptors is produced by both estradiol and diethylstilbesterol (10^{-7}-10^{-8} M). Kinetic results on the interaction of prolactin with the microsomal fraction are consistent with a bimolecular reaction involving significant structural rearrangements during the reaction (not diffusion controlled). Stopped flow kinetic measurements on the reaction between estradiol and cytosol receptors show that at low temperatures, the reaction goes in two distinct steps separable in time. The second step may be the reaction found by others (utilizing sedimentation velocity methods), and which precedes translocation of the hormone-receptor complex to the nucleus.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/13/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $90,000

CONTRACT RESEARCH SUMMARY

Title: Effects of Nucleic Acid Preparations on Biological Properties of Mammary Cancer

Principal Investigator: Aaron Bendich, Ph.D.
Name/Address Sloan-Kettering Institute for Cancer Research
Performing Organization: New York, New York 10021

Contract Number: NO1-CB-43904
Starting Date: 6/28/74 Expiration Date: 3/27/77

Goal: To determine the relationship between transfer of nucleic acid preparations into mammary cells and possible changes in their growth potential.

Approach: To ascertain: a) the effect of nucleic acids or nucliec acid-containing organelles on the biological properties of normal and malignant cells, especially those of animal and human origin; b) the possible expression of embryonal or other genes after nucleic acid-cell interaction, especially when animal or human sperm is used as the DNA-containing moiety: c) the acquisition of, or change in level of, malignant potential of the target cells, especially those derived from the mammary gland; d) the refinement or development of methods to achieve a, b, and c.

Progress: It has been found that the treatment of "normal" cells with Cytochalasin B leads only to a binucleate condition (i.e., a single endoreduplication), but after transformation with carcinogens or spermatozoa, multinucleates appear or predominate. Human breast tumor HBT-39 and SH-2 cells also show this effect. The technique may prove useful in evaluating oncogenic potential of cells and in following effectiveness of clinical or in vitro treatments. Human and animal sperm penetrate the breast tumor cells, as observed by light and scanning electron microscopy and by the fluorescence seen after acridine orange staining. The tumor cells were also treated with DNA from normal human and animal sources and the effects are being compared with those after sperm treatment. Cells from normal human breast tissues have been grown in vitro and outgrowths consisting of epithelioid cells were picked for transformation studies with sperm, and for propagation.

Significance for Cancer Research (NCP Objective 2,2 Approach 3,3)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/22/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $85,000

CONTRACT RESEARCH SUMMARY

Title: Role of Stroma in Neoplasia of the Mammary Gland

Principal Investigator: Merton R. Bernfield, M.D.
Name/Address Stanford University
Performing Organization: Stanford, California 94305

Contract Number: NO1-CB-53903
Starting Date: 4/15/75 Expiration Date: 4/14/77

Goal: To define the role of the basal lamina, its constituent glycosaminoglycan (GAG), and specific stromal tissue in mammary morphogenesis occurring postnatally and during pregnancy.

Approach: Micro-dissection, histochemistry, isotopic incorporation and chemical characterization of mouse mammary gland undergoing morphogenesis will be used to:

1) Establish the presence of GAG within the epithelial basal lamina.
2) Elucidate the pattern of basal laminar GAG synthesis, distribution, and turnover and relate this pattern to epithelial morphogenesis.
3) Develop procedures for isolation of epithelial free of stroma but retaining the basal lamina.
4) Establish whether epithelial morphogenesis is dependent upon the basal lamina and specific stromal tissue.

Progress: C3H mouse mammary glands were examined for the presence of basal laminar glycosaminoglycans (GAG) by histochemical and autoradiographic methods. Histochemical evidence for GAG within the epithelial basal lamina was observed by staining with Alcian Blue at 0.3 M $MgCl_2$. Whole mounts of adult virgin glands showed extensive lobulo-alveolar proliferation and less identifiable GAG on these epithelia. After 2 hours in culture there was little incorporation of ^3H-glucosamine into the lamina of glands, while after 20 hours the surfaces of ducts from virgin animals showed more label than at the end bulbs. Glands of 10-day pregnant animals showed more label at the surface of alveoli than at ducts. Purified collagenase was prepared and will be used to remove mammary stroma from epithelium without altering the basal lamina. Previous experiments have shown that a purified fraction could remove mesenchyme of mouse embryonic sub-mandibular glands without altering the basal lamina. Postnatal mammary epithelial basal lamina appear to be similar to embryonic epithelial laminae in containing GAG. Thus the purified collagenase preparations may allow isolation of mammary epithelia retaining the lamina.

Significance for Cancer Research (NCP Objective 4 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Visit Date: 9/5/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $89,000

CONTRACT RESEARCH SUMMARY

Title: Biochemical Means by Which Effector Molecules Bind to Mammary Cell Surfaces

Principal Investigator: Zoltan Lucas, M.D.
Name/Address Stanford University
Performing Organization: Stanford, California 94305

Contract Number: NO1-CB-33905
Starting Date: 5/24/73 Expiration Date: 5/23/77

Goal: To study natural mechanisms which impair the host's cellular immune response to syngeneic mammary tumors.

Approach: Determine the immune response to several rat mammary tumors in syngeneic animals in which the tumors grow progressively. Determine if tumor growth is associated with factors blocking either or both cellular and humoral immunity, investigating both serum (tumor membrane antigen, enhancing or "blocking" antibody, antigen-antibody complexes, and "anti-antibody-to-tumor antigen") and cellular (suppressor T- and suppressor B-cells) factors as well as non-membrane tumor products non-specifically inhibiting the immune response.

Progress: Fischer rats injected with ascitic syngeneic mammary tumor 13762 develop a cellular immune response that parallels that of allogeneic animals in which the tumors regress. The decrease in spleen cytotoxicity occurs after the peak response (on the 8th day), correlating with the appearance of "suppressor" cells. The decrease can be explained by the three-day half-life of differentiated cytotoxic cells and blockage of conversion of memory cells to differentiated cytotoxic cells. Current attempts to separate "cytotoxic," "memory" and "suppressor" cells have met with no success.

We are unable to detect humoral immunity to this tumor using assays for cytotoxic antibodies and antibody-directed cellular cytotoxicity. However, serological blocking factors, presumably immunoglobulins, are detected in three test systems: a) ^{3}H-thymidine uptake in micro mixed tumor-lymphocyte interactions, b) in vitro generation of cytolytic cells, and c) effector assay of either in vitro or in vivo generated cytolytic cells. Serum from rats injected i.p. with 10^{7} tumor cells one to ten days earlier inhibits the first two "sensitization" phase assays 50-100%. However, serum from sham-controls obtained one and two days post-immunization blocks non-specifically. None of the sera inhibited ^{3}H-thymidine uptake following PHA stimulation. The blocking factor(s) is currently being characterized biochemically.

Significance for Cancer Research (NCP Objective 4 Approach 1)

Project Officer: D. Jane Taylor, Ph.D., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/12/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: Approx. $130,500

CONTRACT RESEARCH SUMMARY

Title: Control of DNA Synthesis in the Mammary Gland

Principal Investigator: Frank E. Stockdale, M.D., Ph.D.
Name/Address Stanford University
Performing Organization: Stanford, California 94305

Contract Number: N01-CB-23875
Starting Date: 6/1/72 Expiration Date: 5/31/77

Goal: To determine the role of serum factors and hormones in the initiation and control of DNA synthesis in mammary gland epithelium and mammary neoplasms of mice.

Approach: Studies include: (1) the isolation, characterization and source of factors in serum responsible for mammary epithelial growth; (2) comparison of the proliferative response of normal and malignant mouse mammary gland epithelium to mammary serum factor (MSF) and hormones; (3) determination of the mode of interaction of MSF and hormones with normal and malignant mammary epithelium in initiation of growth.

Progress: There is a factor in serum which initiates metabolic events leading to DNA synthesis in normal mouse mammary epithelium and in mouse mammary tumors. The factor bands at pH 5.8-6.2 on isoelectric focusing and has been purified 300-500 fold. The activity resides in a fraction containing two major and three minor proteins on polyacrylamide gel electrophoresis. Molecular weight determinations by gel filtration under acidic conditions indicate an approximate molecular weight of 14,000. The factor is found in sera of many species, with rodent sera being most active. Serum levels of MSF are related to pituitary function, but the factor itself is not prolactin or growth hormone. There is a 50% loss of serum activity following hypophysectomy and injections of growth hormones, prolactin or estrogen into hypophysectomized animals does not restore activity to the sera. The growth response to mammary serum factor or to insulin are differentially affected by the developmental stage of the mouse mammary epithelium (MME). As the epithelial population changes as animals advance in pregnancy to lactation, MME becomes increasingly insensitive to MSF but retains the initial level of insulin sensitivity. These data may suggest that the mammary gland is composed of at least two cell populations or that there are changes in the reactive site for MSF within a single cell population. Studies of CFZ mammary tumors in mice show (1) tumor cells are less responsive to MSF than is normal mammary epithelium, (2) tumor cells are as responsive to insulin as is normal epithelium and (3) medium conditioned by tumor cells exhibits mammary serum factor-like activity. These observations suggest that tumor cells may lack MSF receptor sites or mechanisms for responding to MSF and/or produce MSF-like growth promoting factors, thus making themselves less responsive to exogenous MSF. Over 70 human sera from patients with metastatic breast cancer have been tested for MSF activity.

Significance for Cancer Research (NCP Objective 5 Approach 6)
Project Officer: D. Jane Taylor, Ph.D., William Kidwell, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 4/10/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: Approx. $90,000

CONTRACT RESEARCH SUMMARY

Title: Study Breast Cancer Transplantability in Nude-Athymic Mice

Principal Investigator: Beppino C. Giovanella, Ph.D.
Name/Address The Stehlin Foundation
Performing Organization: Houston, Texas 77002

Contract Number: NO1-CB-33856
Starting Date: 2/1/73 Expiration Date: 1/31/77

Goal: To ascertain whether a thymusless-nude strain of Swiss mice can be used as transplant-recipient of human tumors for a study of their growth potential, hormone responsiveness, and their response to chemotherapeutic agents.

Approach: A colony of thymusless-nude Swiss mice has been developed and the good health of the animals permits their use in long-term experiments. Approximately 50 human mammary carcinomas will be transplanted each year, each tumor sample is transplanted into at least 3 females and 1 male. When enough material is available, it will be transplanted also in splenectomized and/or AL(B)S-treated mice and in ones older than 6 months. After transplantation the host will be followed until death. If the tumor grows, it will be retransplanted for 3 generations if feasible. The transplanted tumor fragment will be characterized by a pathologist. The morphology and karyotype of the growing mass will be compared with the original fragment.

Progress: Ninety-three primary and metastatic human breast carcinomas and 16 cell lines from human breast cancers have been transplanted in 842 nude-thymusless mice. Of 57 breast cancers transplanted directly from the patient into untreated nudes and observed for 90 days or more, 28 grew slowly. Many regressed, some reappearing after months of latency. Four tumors showed indefinite growth between 6 months and a year. A fifth primary carcinoma grew rapidly and has been transplanted serially; this infiltrating duct cell carcinoma came from a young, premenopausal woman. Eleven human breast cancer cell lines grew vigorously in the nudes. Two of these tumors metastasized to lymph nodes and lungs. Two cell lines exhibited preferential growth in males, two in females. Of 30 tumors transplanted into AL(B)S-treated mice, 13 grew, 8 of them indefinitely. Four tumors so obtained, together with one which developed in untreated mice and nine obtained injecting cultured cell lines, have already been successfully retransplanted at least once.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: D. Jane Taylor, Ph.D., Harold Hoffman, Ph.D
Program: Breast Cancer Experimental Biology Site Visit Date: 10/27/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $148,000

CONTRACT RESEARCH SUMMARY

Title: Microcirculation and Molecular Transport in Mammary Carcinomas

Principal Investigators:	Joseph F. Gross, Ph.D.,	Marcos Intaglietta, Ph.D.
Name/Address	University of Arizona	University of California
Performing Organization:	Tucson, Arizona 85721	San Diego, California 92037

Contract Number: NO1-CB-63981
Starting Date: Expiration Date:

Goal: To study the local velocity and pressure in single vessels and quantitate the movement of materials across the endothelium into the extravascular tissue in tumor microcirculation.

Approach: Fit Fischer 344 rats with a modified algire access chamber containing the BL-DMBA mammary adenocarcinoma for analyses of microcirculatory parameters. Run similar studies using human mammary adenocarcinoma specimens with the algire chamber implanted into nude-athymic mice. Obtain morphological data by planimetric techniques by measuring the diameter and length of developed two dimensional microvasculature. On microvasculature developed into a three dimensional configuration infuse intravenously fluorescein tagged albumins and photograph the microvasculature through the image intensifier. Determine blood flow by measuring the velocity of red blood cells using the dual slit technique. Determine the capillary pressure by inserting a micropipette into the vessel. Characterize the diffusion and transport in the extravascular compartments by analyzing the extravasation of dyes injected intravenously or intra-arterially. Develop analytical models for characterizing microcirculation of tumor systems.

Progress: New Contract

Significance for Cancer Research (NCP Objective 4 Approach 1)

Project Officer:
Program: Breast Cancer Experimental Biology Site Visit Date: 2/5/76
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: Approx. $97,807

CONTRACT RESEARCH SUMMARY

Title: Mammary Gland Responsiveness to Multiple Hormones

Principal Investigator: R. David Cole, Ph.D.
Name/Address University of California
Performing Organization: Berkeley, California 94720

Contract Number: NO1-CB-43866
Starting Date: 6/29/74 Expiration Date: 6/28/77

Goal: Determine mechanism(s) underlying the ability of the mammary gland to respond to several steroid and peptide hormones.

Approach: 1) Demonstrate hormone sensitivity in mouse mammary epithelial cell line (NMuMG). 2) Observe hormonal effects on the program of chromatin assembly through the sequence of appearance and modification of chromosomal proteins. 3) Observe maturation of chromatin by characterizing 3 or 4 structural categories of chromatin fragments at different stages of chromatin maturation. 4) Compare all aspects of above with other mammary tumor cell lines to NMuMG. 5) Assay for prolactin receptors.

Progress: The NMuMG cells were tested for responsiveness to hormones: insulin; cortisol or dexamethasone; and prolactin. The cells were found to possess 8,000 membrane bound insulin receptors and 20,000 cytoplasmic glucocorticoid receptors per cell, but the steroid receptors could be demonstrated only with the use of serum tested with charcoal. Extraction of endogenous hormone was done at 37°C because high temperature destroys the ability of the serum to support cell growth. Cells grown with charcoal-treated (37°) serum grow at essentially normal rates but a combination of insulin, dexamethasone and prolactin enhances growth in the earliest stages after plating the cells, perhaps by increasing plating efficiency. Also, about 7 days after confluencey is reached the hormone combination produces many large or flat cells that are stable for at least 2 weeks. Steroid alone produces fewer such cells and more slowly, while insulin treatment has no effect.

Assays of the NMuMG cells for lactose synthetase appears to show a wave of induction of both A and B subunits of the enzyme when cells are treated with insulin, glucocorticoids and prolactin. A partial effect is shown with insulin alone. The magnitude of the enzyme induction is three to four fold. Insulin induces a doubling of the activity of glyceraldehyde phosphate dehydrogenase.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., E. Brad Thompson, M.D.
Program: Breast Cancer, Experimental Biology Site Visit Date: 3/13/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: Approx. $60,000 Terminal

CONTRACT RESEARCH SUMMARY

Title: Hormones Required for Normal and Abnormal Human Mammary Tissue in Organ Culture

Principal Investigator: Joel J. Elias, Ph.D.
Name/Address University of California
Performing Organization: San Francisco, California 94143

Contract Number: N01-CB-23861
Starting Date: 6/19/72 Expiration Date: 6/18/76

Goal: 1) To find conditions to maintain growth of normal, dysplastic and neoplastic human mammary tissue in organ culture. 2) To see whether morphologic classification of human mammary lesions can be improved so as to be of prognostic value.

Approach: Human mammary tissue from biopsies, mastectomies, and mammoplasties will be utilized for the following studies: 1) Electron microscopic, scanning electron microscopic and light microscopic preparations of normal and abnormal human mammary tissue to define histological, structural and surface characteristics. 2) Organ culture and eventually cell culture of material studied in (1). Analysis of conditions necessary for promoting growth. 3) Effects of hormones on organ cultures studied with EM, SEM and LM. Correlation with biological behavior in vivo.

Progress: Organ culture techniques were developed for human breast carcinomas to be maintained up to 20 days in medium 199. Tritiated thymidine labeling and autoradiography showed minimal DNA synthesis in explanted carcinoma cells. Attempts to stimulate DNA synthesis using various hormone combinations and concentrations, placental cord serum, and serum from the patient were unsuccessful. Attempts to stimulate ductal or alveolar growth and/or secretion in dysplasias in culture by using various combinations of insulin, cortisol, human placental lactogen, etc., have not been successful. Progress increased in recognizing surface features of epithelial cells in human mammary dysplasias, fibroadenomas and carcinomas by scanning electron microscopic (SEM) methods. Tissue from biopsy or mastectomy from 43 women were studied be SEM to date. Analysis of specimens from the first 13 women easily identified infiltrating ductal carcinoma cells and, more so, recognized that duct epithelium within breasts containing benign lesions differed from that within carcinomatous breasts. Characteristics of duct cells in carcinomas are (1) partitioning of surface microvilli into small groups of 3 or more clumped together at the tips; (2) microvilli from adjacent cells touching at their apices; (3) prominent clump of microvilli-like projections in the center of apical surface. Ducts from histologically "normal" areas of the breast and as far as 7 inches from biopsy site showed these structural characteristics.

Significance for Cancer Research (NCP Objective 4 Approach 1)

Project Officer: D. Jane Taylor, Ph.D., Katherine Sanford, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/9/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: None; Terminal

CONTRACT RESEARCH SUMMARY

Title: Neoplastic Potential of Preneoplastic Lesions of the Human Mammary Gland

Principal Investigators: S.R. Wellings, M.D.
Hanne Jensen, M.D.
Name/Address Performing Organization: University of California Medical School
Davis, California 95616

Contract Number: NO1-CB-43908
Starting Date: 6/28/74 Expiration Date: 6/27/77

Goal: Define the growth potential of hyperplastic-dysplastic lesions of the human mammary gland also termed "preneoplastic" or "latent cancer cells."

Approach: Analysis of the growth potential of human mammary heterotransplants in athymic mice. Transplants of ducts, lobules, carcinomas *in situ* and evident carcinomas will be implanted into female and male mice. The site of the implant will be cleared fat pad as well as intraperitoneal and subcutaneous. Also some transplants will be done with primary cultures following brief *in vitro* cultivation of the lesions. Vascular and inflammatory response of the host to the transplants will be determined and the difference between biopsy and autopsy material will be assessed. The hormonal milieu of the host will be manipulated in reference to growth capacity of transplants.

Progress: Twenty mastectomy and 26 biopsy specimens provided 292 transplants into nude-athymic mice. Most transplants were normal appearing lobules. Transplants 3-6 mm in diameter survived best. The cleared fat pad was the best site whereas the subcutaneous site was suboptimal and intraperitoneal site the poorest. Transplants into young female mice evoked a greater vascularization than those in older or in male mice. *In vitro* maintenance prior to transplantation caused some dedifferentiation. Six transplants of normal appearing lobules from 2 mastectomy specimens underwent dedifferentiation after 6 months *in vivo* of which 85% were in the cleared fat pad and the rest in the subcutaneous site. Of all transplants, 73% were in female and 27% in male mice; 80% were in the subcutaneous site, 8% in cleared fat pad and 12% in the intraperitoneal site. Sixty-nine percent of the transplants were found at autopsy, of which 46% were vasularized and 43% were well perserved 3 weeks to 6 months after transplantation. Very few atypical lobules were found to transplant. More mastectomy specimens from women aged 40-50 will be examined which may yield more atypical lobular lesions.

Significance for Cancer Research (NCP Objective 4,5 Approach 2,4)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Date Visit: 3/12/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: Approx. $149,970 Terminal

CONTRACT RESEARCH SUMMARY

Title: Biochemical Nature of Hormone Dependency in Breast Cancer

Principal Investigator: Eugene DeSombre, Ph.D.
Name/Address University of Chicago
Performing Organization: Chicago, Illinois 60637

Contract Number: NO1-CB-23873
Starting Date: 6/1/72 Expiration Date: 5/31/77

Goal: To assess the role of hormones and specific hormonal receptors in the control of tumor growth.

Approach: Carcinogen-induced (DMBA) primary or transplantable mammary tumors of rats and appropriate control tissues are utilized. Major emphasis is to be placed on tumor RNA polymerase activity as affected by hormones; however, other projects on hormonal control of tumor growth and the role of hormonal receptors are to be continued. Major areas of study are: tumor RNA polymerase (I and II) activity; hormone receptors in apparently autonoumous mammary tumors; interrelation of estrogen and prolactin receptors in tumors; transplantable, hormone-dependent mammary tumors; regulation of tumor peroxidase activity; and preliminary studies to determine whether antibody to purified uterine estrogen receptor will cross react with mammary tumor estrogen receptors.

Progress: Before assaying for RNA polymerase stimulation by estrogen the tumor is classified as hormone-dependent (HD) or independent (HI) according to in vivo tumor response following castration. Assay is in a low salt Mg^{++} system, is dependent on all 4 ribonucleotide precursors and is inhibited by actinomycin D. At 30°C RNA synthesis is linear for 5-10 minutes with no appreciable difference in product degradation in hormone-treated and control groups. Incubation of tumor homogenates with estradiol (E2) stimulates RNA polymerase I activity in hormone-dependent tumors by not with hormone-independent tumors. Requirements for E2 stimulation are receptor containing cytosol and HD tumor nuclei. Anti-estrogen (CI628) which effects regression of HD tumors in vivo appears to block E2-induced stimulation in vitro. Some but not all HI tumors have significant concentrations of estrogen receptors. Mammary tumor peroxidase, characteristic of HD tumors, disappears after ovariectomy but reappears after E2 administration in vivo. Subcellular distribution of peroxidase is characteristic of tumor cell synthesis rather than cell uptake. Correlation of tumor concentrations of both prolactin and estrogen receptors shows most HD tumors have high levels of both receptors and HI tumors generally have low levels of both. Some HD tumors have elevated levels of only one receptor implying a spectrum of dependences, some on both hormones, some on only one of the two hormones.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 4/12/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: Approx. $120,000 Terminal

CONTRACT RESEARCH SUMMARY

Title: Osteotropism of Mammary Carcinoma Metastasis

Principal Investigator: Gregory R. Mundy, M.D.
Name/Address University of Connecticut Health Center
Performing Organization: Farmington, Connecticut 06032

Contract Number: NO1-CB-63980
Starting Date: Expiration Date:

Goal: To identify the mechanisms by which bone is altered in breast cancer.

Approach: 1) Determine the cellular mechanism of bone destruction in metastatic breast cancer. 2) Determine the hormonal factors responsible for hypercalcemia and/or bone metastasis in breast cancer. 3) Characterize the effects of these factors on bone and identify ways in which those effects can be prevented or opposed. 4) Investigate pharmacological inhibition of the bone resorbing factors produced by breast cancer cells. 5) Investigate the production of substances which stimulate new bone formation and collagen synthesis in breast cancer. In vitro and in vivo systems of human and animal models are utilized.

Progress: New Contract

Significance for Cancer Research (NCP Objective 4 Approach 1)

Project Officer:
Program: Site Visit Date: 2/19/76
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: Approx. $104,249

CONTRACT RESEARCH SUMMARY

Title: Structure Function Relationship of Prolactin Interactions in Mammary Gland Cells

Principal Investigator: Kurt E. Ebner, Ph.D.
Name/Address University of Kansas Medical Center
Performing Organization: Kansas City, Kansas 66103

Contract Number: NO1-CB-63985
Starting Date: Expiration Date:

Goal: To modify ovine prolactine and determine the critical amino acids involved in binding of the hormones to membranes, activation of adenyl cyclase and stimulation of α-lactalbumin formation.

Approach: Modify prolactin chemically by using: mercaptoethanol, N-ethylmaleimide, iodoacetamide, cyanogen bromide, tetranitromethane, carbodiimide. Use trypsin, chemotrypsin, plasmin, pepsin and leucine amino acid peptidase for enzyme modification of prolactin. Modifications will include amino acids: (tyrosin, tryptophan, lysine, methionine, histidine), carboxyl groups and reduction of disulfides. Prolactin activity will be assayed by binding to mammary particles, ability to activate adenyl cyclase in mammary plasma membranes and stimulate α-lactalbumin synthesis of mid-pregnant mammary explants.

Progress: New Contract

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer:
Program: Breast Cancer Experimental Biology Site Visit Date: 2/2/76
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: Approx. $55,498

CONTRACT RESEARCH SUMMARY

Title: Study of Mammary Gland Responsiveness to Multiple Hormones

Principal Investigator: William Pearlman, Ph.D.
Name/Address University of North Carolina
Performing Organization: School of Medicine
Chapel Hill, North Carolina 27514

Contract Number: NO1-CB-53905
Starting Date: 4/1/75 Expiration Date: 3/31/77

Goal: To search for the presence of steroid hormone-binding proteins or receptors in colostrum and milk obtained from humans and animals, and to characterize or identify the specific steroid-binding components.

Approach: (1) Studies on humans. The whey from pre- and post-partum colostrum and milk obtained at progressive stages of lactation will be assayed for binding activity with respect to progesterone, cortisol, 17β-estradiol and testosterone. They whey proteins will be subjected to various fractionation procedures so that the protein components responsible for specific steroid-binding activity may be purified and partially characterized or identified by physicochemical methods. (2) Studies on animals. The lactating rat will be studied similarly so that a suitable animal model may be developed to ascertain the effect of multiple hormonal stimulation (e.g., with 17β-estradiol, progesterone, and cortisol, singly or in combination) on the nature and level of steroid-binding activity in colostrum and milk. The steroid-binding activity of colostrum and milk from other animal species, particularly the cow, will be studied for comparative purposes.

Progress: We have found a progesterone- and cortisol-binding component resembling serum corticosteroid-binding globulin in pre- and post-partum colostrum and late milk obtained from women and in milk obtained from lactating rats. The lactating rat thus appears to be a suitable animal model for further investigation. This research work was begun just prior to the initiation of the contract and immediately following.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Visit Date: 7/24/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $89,400

CONTRACT RESEARCH SUMMARY

Title: Studies of Isoenzymes in Normal, Preneoplastic and Neoplastic Breast Tissues

Principal Investigator: Russell Hilf, Ph.D.
Name/Address University of Rochester
Performing Organization: Rochester, New York 14642

Contract Number: NO1-CB-33880
Starting Date: 5/21/73 Expiration Date: 8/20/76

Goal: To examine multiple molecular forms of glucose-6P-dehydrogenase (G6PD) and lactate dehydrogenase (LDH) to characterize normal, preneoplastic and neoplastic mouse mammary tissue.
Approach: Molecular forms of G6PD and LDH to be examined in normal mouse mammary tissue (pregnancy, lactation, parturition) and in preneoplastic and neoplastic tissue exposed to the same hormonal milieu in vivo. Determine sulfhydryl content of tissues, with emphasis on reduced glutathione, to seek correlations with G6PD profiles in normal and abnormal tissue. Establish the molecular weights of G6PD-III, II and I, using methods such as that of Hedrick-Smith. Purify and characterize G6PD from glands of lactating mice. Use G6PD as an antigen to induce antibodies. Expand studies on G6PD forms in tissues of mice possessing NIV and MTV.
Progress: Enzyme activities (per mg protein) in hyperplastic alveolar nodules (HAN) from virgin mice were more like those of mammary glands (MG) from pregnant mice than mammary tissue (MT) from lactating mice. However, when expressed per mg/DNA they were lower in HAN than in MG from pregnant or lactating mice. In transplanted carcinomas activities resembled those in HAN more so than those in spontaneous tumors or tumors arising from transplanted HAN. Glutathione reductase (GR) activity was elevated in HAN and tumors from virgin mice as compared to MG, whereas the α-glycerolphosphate dehydrogenase activity was lower in both abnormal tissues. HAN from lactating mice showed increase in RNA levels and a decrease in LDH. GPI and GR compared to HAN from virgin mice which suggests preneoplasia retains some ability to respond to altered hormonal milieu. MT from lactating BALB/c mice showed up to 50% of the slow migrating G6PD-III which is absent in glands from pregnant mice, HAN, and carcinomas. All tissues possessed the faster migrating G6PD-II, 85% in pregnant MG. The fastest migrating G6PD-I, up to 35% of total activity in HAN and neoplastic tissue, but only 15% in glands from pregnant mice and absent in lactating gland. Addition of DTT to tissue did not alter G6PD activity but increased relative activity of G6PD-II and GPD-I. Molecular weight estimations were 118,000 for G6PD-II and 260,000 for G6PD-III. Progress has been achieved in distinguishing normal from preneoplastic and neoplastic mammary tissue.

Significance for Cancer Research (NCP Objective 4 Approach 1)
Project Officer: D. Jane Taylor, Ph.D., Harold Hoffman, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 6/21/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: Extended without funds.

CONTRACT RESEARCH SUMMARY

Title: Alteration of Mammary Neoplastic Cells by Manipulation of Cellular Environment

Principal Investigator: Greed W. Abell, Ph.D.
Name/Address The University of Texas Medical Branch
Performing Organization: Galveston, Texas 77550

Contract Number: NO1-CB-43906
Starting Date: 6/10/74 Expiration Date: 6/[illegible]/77

Goal: To manipulate the cellular environment in vivo and in vitro in order to limit preferentially the growth of neoplastic cells of mammary origin.

Approach: To evaluate the effects of phenylalanine ammonia-lyase (PAL) and phenylalanine restriction on mammary tumors in mice and rats and on human breast tumors growing in athymic mice. Several PALs, obtained from different sources or modified chemically to decrease their rates of clearance from the host, will be studied and compared for therapeutic effectiveness.

Progress: PAL, isolated from the yeast Rhodotorula glutinis, has been purified to homogeneity and evaluated against the BW10232 mammary tumor in C57 black mice. PAL significantly inhibited tumor growth during a 5-day period of treatment. Diets restricted in phenylalanine inhibited tumor growth but also caused appreciable weight loss of the the host. Following treatment with PAL, phenylalanine and tyrosine levels in the blood of mice (with or without tumor) and other species were reduced to less than 10% of the control in 1 hour. The clearance of PAL from the blood was demonstrated by enzymic assay and by cross-reactivity with an antibody to PAL (raised in rabbits). Following a single injection, the enzyme was cleared with a half-life of about 36 hours, whereas with repeated administration (14 days), the enzyme had a half-life of about 4 hours. Interestingly, no precipitating antibody could be demonstrated after repeated administration of PAL. Since PAL caused a temporary inhibition of tumor growth, studies were initiated with PAL and phenylalanine mustard in an attempt to increase therapeutic effectiveness.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/14/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: Approx. $96,067

CONTRACT RESEARCH SUMMARY

Title: Biochemical Mechanism of Endocrine-Induced Breast Cancer Regression

Principal Investigator: William L. McGuire, M.D.
Name/Address University of Texas Health Science Center
Performing Organization: San Antonio, Texas 78284

Contract Number: NO1-CB-23862
Starting Date: 6/1/72 Expiration Date: 5/31/77

Goal: To identify those breast cancer patients whose disease will respond to hormonal manipulation and to obtain new knowledge of the hormonal controls on tumor growth.

Approach: Development of predictive procedures for hormone dependence in human breast cancer by extending estrogen receptor (ER) clinical studies into new areas, by simplifying ER assays, and by investigating other steps in hormonal control to gain additional information on hormone responsiveness. In addition to ER determinations on human breast cancer tissue, we will undertake studies of prolactin (Prl R) and progesterone receptors (PgR).

Progress: Several hundred human breast tumors assayed for ER; more than 50 have been objectively evaluated for clinical response to endocrine therapies. Tumors without ER had 100% negative response whereas ER positive tumors had 60% objective regression. Methodology for determining ER was improved by use of thiol reagents and temperature control. Solid phase ligand exchange assay was developed to determine ER in the presence of endogenous estrogen. To better understand ER+, endocrine therapy failures we chose progesterone receptor (PgR) as a new biochemical marker of an intact estrogen response system. Of 24 ER- tumors, 0% contained PgR, whereas of 76 ER+ tumors, only 54% contained PgR, similar to the expected response rate. Where clinical response can be compared directly, 3/3 ER+, PgR+ tumors had objective regressions, 4/4 ER+, PgR- tumors failed to respond, and 5/5 ER-, PgR- tumors failed to respond. The anti-tumor agent estradiol mustard (EM) has low affinity for ER *in vitro* and a long time lag before effects on ER *in vivo* were observed which suggests a hydrolysis product and not EM is responsible. Methodology for assaying prolactin receptors in experimental tumors now being extended to human breast tumors to correlate with ER and PgR. Investigation *in vivo* metabolism of ^{125}I-estrogens to develop non-invasive technique for measuring tumor ER.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: D. Jane Taylor, Ph.D., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/24/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: Approx. $120,000

CONTRACT RESEARCH SUMMARY

Title: Differentiation of Mammary Epithelial Cells

Principal Investigator: Howard L. Hosick, Ph.D.
Name/Address Washington State University
Performing Organization: Pullman, Washington 99163

Contract Number: NO1-CB-63986
Starting Date: Expiration Date:

Goal: To study how several mesenchymal cell types in the adult mouse mammary gland interact with mammary epithelium to regulate its development.

Approach: Mid-pregnant type mammary tissue will be obtained from C3H mice implanted with pellets containing estrogen and desoxycorticosteroids. Isolate epithelial cells using collagenase or pronase, Ficoll gradient centrifugation and medium containing the D-isomer of valine. Incorporate ^{3}H-oleate or ^{14}C-oleate into the cells by using liposomes. Grow cells on artificial capillaries and study the uptake of fatty acids supplied from within the capillary. Measure the growth responses to fatty acids by: 1) changes in cell number: 2) changes in rate of DNA accumulation; 3) changes in rate of protein accumulation.

Progress: New Contract

Significance for Cancer Research (NCP_____Approach_____)

Project Officer:
Program: Breast Cancer Experimental Biology Site Visit Date: 2/4/76
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: Approx. $28,065

CONTRACT RESEARCH SUMMARY

Title: Studies on Role of Prostaglandins in Mammary Gland Neoplasia

Principal Investigator: Sumner Burstein, Ph.D.
Name/Address Worcester Foundation for
Performing Organization: Experimental Biology
Shrewsbury, Massachusetts 01545

Contract Number: NO1-CB-33920
Starting Date: 6/1/73 Expiration Date: 2/29/76

Goal: To determine the role of prostaglandins (PG) in mammary gland neoplasia.

Approach: Primary monolayer cultures of epithelial cells from normal and tumor-bearing mice to be used as the model system. Mammary cells of human origin will be utilized when they become readily available. Studies of PG synthesis in cultured cells, normal and neoplastic, from pregnant, lactating and tumor-bearing C_3H mice to continue. Factors affecting PG synthesis, inhibitors and stimulators, will be examined. In collaboration with Dr. Maudsley, studies of effects on cyclic nucleotide synthesis will be expanded.

Progress: Developed primary cultures of mammary epithelial cells into a viable and practical system for the study of PG synthesis of the mammary gland. Observations of PG synthesis in cultured cells have shown that arachidonic acid (20:4) is a more efficient precursor for PG production than eicosatrienoic acid (20:3). Dibutyryl cAMP was found to stimulate the synthesis of PGE_1 and PGF_{1a} in normal cells. There appeared to be a tissue-dependent shift from stimulation of PGE at 49 hours to PGF at 72 hours and also a reduction of phospholipid and an increase in triglyceride content of the cells. C_3H mouse mammary tumor cells have not been tested to date with dibutyryl cAMP. However, the tlc patterns of tumor cells did not seem to differ significantly from normal cells. They were found to be unresponsive to LH, phospholipase A and drugs such as Naproxen and CBN. Utilizing RIA techniques PGE and PGF production has been monitored. Basal levels were raised by collagen and lowered by Naproxen. RIA studies will be continued to examine other aspects such as plating density effects, time course, etc.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., William Kidwell, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 5/3/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: None; Terminal

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Mammary Epithelial Cell Plasma Membrane

Principal Investigator: Grant Fairbanks, Ph.D.
Name/Address Worcester Foundation for
Performing Organization: Experimental Biology
Shrewsbury, Massachusetts 01545

Contract Number: NO1-CB-33908
Starting Date: 6/1/73 Expiration Date: 2/29/77

Goal: Development of new methods for plasma membrane isolation and characterization applicable to normal and neoplastic mammary cells.
Approach: Novel radiolabeled reagents for cell surface modification will be synthesized. The altered physical or chemical character of plasma membranes from modified cells will be the basis for membrane purification. Ascites sublines of the 13762 rat mammary adenocarcinoma will serve as a test system for the reagents. The methodology will be applied to the human cell lines established from normal and malignant mammary tissue.
Progress: In the first radiosynthesis of a cleavable protein cross-linking reagent, ^{35}S-dithiobis(succinimidyl propionate) (^{35}S-DTSP) was prepared at 20 mCi/mmol. DTSP rapidly acylates protein amino groups. It reversibly cross-links proteins when applied for 1-5 minutes at 0°, pH 7 at concentrations below 0.1 mM. Cells of 13762 ascites sublines briefly treated with DTSP are highly resistant to hypotonic and shear stresses. Membrane fractions from treated cells were highly enriched in protein-bound radioactivity derived from ^{35}S-DTSP. The results indicate that DTSP will be useful in probing protein organization on the mammary cell surface.

Pyridoxamine phosphate (PMP) was condensed with ^{35}S-DTSP to yield a new reagent for plasma membrane labeling and isolation. Affinity columns bearing apo-aspartate aminotransferase will be used to selectively adsorb plasma membranes derivatized with the PMP-DTSP reagent; in pilot studies the adsorbent has been shown to bind the reagent.

Treatment of aminoalkylated glass with ^{35}S-DTSP yielded activated beads that couple covalently, but reversibly, to amino groups on cell surfaces. Activated bead will be used for labeling and rapid isolation of surface proteins. Extracellular nucleases interfered with the uses of ribonucleotide homopolymers as membrane isolation reagents. Analysis of spontaneous binding of ^{3}H-Poly(U) to surfaces of 13762 ascites tumor cells revealed that a minor population of very small cells is responsible for binding, which can be prevented or reversed by treating cells with purine nucleosides or pyrimidine bases.
Significance for Cancer Research (NCP Objective 3 Approach 5)
Project Officer: D. Jane Taylor, Ph.D., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 5/3/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $50,000

CONTRACT RESEARCH SUMMARY

Title: Role of 3', 5'-cAMP and Related Enzyme Systems in Mammary Neoplasia

Principal Investigator: David Maudsley, Ph.D.
Name/Address Worcester Foundation for
Performing Organization Experimental Biology
Shrewsbury, Massachusetts 01545

Contract Number: NO1-CB-33919
Starting Date: 6/1/73 Expiration Date: 11/3/76

Goal: To study the role of cyclic adenosine 3', 5'-monophosphate (cAMP) in the mammary gland tissue, normal and abnormal, and to establish whether the incidence of mammary tumors alters cAMP metabolism.

Approach: The intitial studies used freshly isolated cell suspensions and primary monolayer cultures of mouse epithelial cells but future studies will be concerned with comparing the findings obtained with the mouse tissues with those of established human cell lines. The effects of prostaglandins and prostaglandin precursors on cAMP production will be studied. Procedures for manipulating endogenous levels of cAMP will be investigated for defining the regulatory role of this component in cell growth.

Progress: Prostaglandin E_1 (PGE_1), epinephrine (EP) and cholera enterotoxin (CT) stimulated cyclic AMP (cAMP) levels in isolated cell suspensions of mammary tissue from mid-pregnant C_3H mice whereas insulin, prolactin, ACTH, and glucocorticoids had no effect. PGE_1 response occurred in 30 seconds and was complete by 20 minutes. CT response occurred within 10 minutes and reached maximum at 90 minutes. Response of all 3 agents was enhanced by theophylline. PGE_1 combined with EP or PGE_1 with CT were additive which suggested that these stimuli operate through different mechanisms or stimulate different cell types. In isolated cell suspensions CT was the most potent, followed by PGE_1 and EP whereas in primary monolayer cultures PGE_1 gave the lowest response. Study suggested a component in cell suspension in addition to epithelial cells was responsive to PGE_1 stimulation. Cell suspensions from rat mammary tumors DMBA, 13762 and R-35 all had similar response profiles to CT, PGE_1 and EP. None of these tumors responded to hormonal stimulation. The ascites 13762A responded to CT and EP but the response to PGE_1 was markedly reduced.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., William Kidwell, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 5/3/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $52,600 Terminal

CONTRACT RESEARCH SUMMARY

Title: Cell Kinetics of Breast Cancer

Principal Investigator: Lewis M. Schiffer, M.D.
Name/Address Allegheny General Hospital
Performing Organization: Pittsburgh, Pennsylvania 15212

Contract Number: NO1-CB-43899
Starting Date: 2/1/74 Expiration Date: 1/31/77

Goal: To develop methods of rapidly determining cell kinetic parameters of individual human breast cancers so that this information may potentially be used in the study of the natural history and treatment of these tumors.

Approach: The spontaneous C3H mammary tumor is used as a model system to develop rapid techniques for totally in vitro estimation of cell cycle kinetics, and subsequent transfer of these techniques to human use. Perturbations of normal kinetics following single and multiple drug chemotherapy are being utilized to time-sequence therapeutic modalities. Similar studies with the transplantable rat 13762 tumor are in progress. In vitro cell kinetics, including the number of cells in, and time of, DNA synthesis, cell cycle time and estimation of growth fraction are now performed in human breast tumors, both primary and metastatic. After the acquisition of a sufficient data base, the perturbation of human tumor cytokinetics by chemotherapeutic drugs will be studied.

Progress: For the C3H tumor the cytokinetics and growth curves were characterized. The in vitro cytokinetic results were found to be identical to control in vivo studies. The results with the rat tumor and human tumors, thus far, also appear to be similar. Primary human tumors appear to have a longer cell cycle time than metastases, mainly as a result of a longer G_1 time period. As in the mouse tumor, the DNA synthesis times of human tumors do not vary over a large range, averaging about 18 hours. The average percentage of cells in DNA synthesis of primary tumors is 4% and the estimated percentage of cells in cycle is about 20%. Preliminary evidence shows that the periphery of tumors have different cell kinetics than the central areas. Individual cytokinetic results are now obtainable in 7 days in all species studied.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: Mary E. Sears, M.D., Stanley Shackney, M.D.
Program: Breast Cancer Treatment Site Visit Date: 10/31/74
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $163,400

CONTRACT RESEARCH SUMMARY

Title: Therapy for Stage II or Stage III Carcinoma of the Breast

Principal Investigator: Charles A. Hubay, M.D.
Name/Address Case Western Reserve University
Performing Organization: Cleveland, Ohio 44106

Contract Number: NO-CB-43990
Starting Date: 6/16/74 Expiration Date: 6/15/77

Goal: To test the effects of adding chemotherapy, anti-estrogen therapy, and BCG to mastectomy in patients with locally advanced breast cancer.

Approach: Patients under age 76 who show axillary nodes involved with metastases at the time of surgery are eligible for this study. Stratification, but not treatment selection, is on the basis of the presence or absence of estrogen receptor protein (ER) in the tumor. Random treatment assignments are 1) cyclophosphamide, methotrexate, 5-fluorouracil (CMF) for 12 months, 2) CMF plus tamoxifen (CMFT) for 12 months, or 3) CMFT for 12 months plus BCG for 12 months. Endpoint is the first evidence of treatment failure, i.e., the appearance of locally recurrent or of distant tumor.

Progress: Patient entry into the study began in September 1974. Accession is faster since the requirement was dropped to 1 or more positive nodes. A recent change is the substitution of CMF for an observation only control arm. There have been no serious problems with toxicity due to treatment. No data are available as yet concerning treatment failure incidence.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment Site Visit Date: 4/1/74
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $150,000

CONTRACT RESEARCH SUMMARY

Title: Study of Hormone Manipulation Plus Chemotherapy in Breast Cancer Patients

Principal Investigator: Melvin Moore, M.D.
Name/Address Emory University School of Medicine
Performing Organization: Atlanta, Georgia 30303

Contract Number: NO1-CB-43916
Starting Date: 6/1/74 Expiration Date: 2/29/76

Goal: To determine whether the incidence and/or duration of anti-tumor effectiveness are increased by combining hormone therapy and chemotherapy.

Approach: Postmenopausal women with their first evidence of metastasis are assigned to one of two treatment regimens: estrogen (E) or estrogen plus cyclophosphamide, adriamycin, and 5-fluorouracil (E-CAF). In those patients assigned to the first regimen (E), chemotherapy is started upon documentation of treatment failure. The patients are observed for objective evidence of tumor regression, duration of remission, and quality of performance status. Efforts are now being made to obtain tissue for estrogen and progesterone receptors on all patients at the time of entry on study.

Progress: As of September 1, 1975, there have been 31 patients entered on study. Eighteen patients randomized to E and 13 to E-CAF (disparity in size of groups is due to the number of stratifications). Twenty-four patients have completed 2 months of therapy and are thus evaluable for response. Among 14 patients in the E group there is one PR, 2 NC (21% PR, NC) and 11 progressors (79%). Among 10 patients in the E-CAF group there are 7 PR, 2 NC (90% PR, NC) and one with progression (10%). Median duration of response to E-CAF will be in excess of 7 months.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment Site Visit Date: Pending
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: None; Terminated

CONTRACT RESEARCH SUMMARY

Title: Therapy of Patients with Stage II or Stage III Carcinoma of the Breast

Principal Investigator: Edward F. Scanlon, M.D.
Name/Address Evanston Hospital
Performing Organization: 2650 Ridge Avenue
Evanston, Illinois 60201

Contract Number: NO1-CB-53917
Starting Date: 6/30/75 Expiration Date: 6/29/77

Goal: To determine the effects of adding chemotherapy and BCG to mastectomy for patients with locally advanced breast cancer.

Approach: Patients who have metastatic axillary nodes or Stage III breast cancers are admitted to the study program. Stratification and randomization are carried out along with a balancing for prognostic factors according to the following variables: Primary tumor size, number of positive nodes, menstrual status and unfavorable local signs. The statistical analysis involves sequential treatment assignment and is designed to provide the greatest balance between all groups assigned according to all available prognostic indicators. One of three treatment schedules is assigned to each of the patients in the study according to the above computer classifications: (1) intermittent phenylalanine mustard (PAM), (2) 5-fluorouracil, cyclophosphamide and prednisone in intermittent courses, and (3) the three drug chemotherapy plus BCG inoculations. Two of the three treatment schedules are identical with the Mayo Clinic adjuvant study and the data will be cumulative. The statistical method of analysis is also identical with that now being used at the Mayo Clinic. The treatment course will be given for one year or until there is evidence for relapse, whichever occurs first. Immunologic evaluation is done initially and every three months. Survival and recurrence free intervals will be computed for each treatment group.

Progress: Thirty-one patients were entered into this study during the first 6 months, equally divided among treatment groups and hospitals. All but five of the patients are postmenopausal and most of these patients have fewer than 4 positive lymph nodes. The initial tolerance of the patients to all three treatment groups has been excellent with minimal side effects and good patient acceptance. There are no data as yet concerning treatment results.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Donald Henson, M.D.
Program: Breast Cancer Treatment Site Visit Date: 8/29/74
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $200,000

CONTRACT RESEARCH SUMMARY

Title: Predictive Transplantable Animal Mammary Tumor Models

Principal Investigator: Arthur E. Bogden, Ph.D.
Name/Address Mason Research Institute
Performing Organization: Harvard Street
Worcester, Massachusetts 01608

Contract Number: NO1-CB-43914
Starting Date: 11/1/73 Expiration Date: 10/31/76

Goal: To carry out preclinical evaluations of single and combined therapeutic modalities in experimental mammary tumors of known biological characteristics.

Approach: Studies are being done in six transplantable rat mammary tumor systems having a spectrum of histological, growth and metastasizing characteristics. The highly metastatic 13762 mammary adenocarcinoma is used most extensively and the R3230AC, SMT-2A, 3M2N, DMBA#1 and DMBA#14 mammary tumors are used to further test and evaluate those drug and therapy combinations showing promising activity in the 13762 system. Chemotherapy assays versus subcutaneous grafts are made by initiating drug treatment on the day following implantation or on day 15 when tumors are well established. Metastasis assays combine surgical extirpation of tumors on day 18 with other therapeutic modalities. The parameters of immunosuppression and hormone radioimmunoassay are applied in studies requiring such in-depth drug and therapy evaluation. Studies on combinations of therapeutic modalities include surgery, chemotherapy, and x-irradiation with and without nonspecific immunological adjuvants. Chemotherapeutic agents of current interest include phenylalanine mustard (PAM), adriamycin (ADR), cytoxan (CTX), 5-fluorouracil (5-FU), methotrexate (MTX), dibromodulcitol (DBC), hexamethylmelamine (HEM), vincristine (VIC), the nitrosoureas and tamoxifen (TAM).

Progress: The response of six mammary tumor systems to PAM alone and in a three drug combination with 5-FU and MTX indicates that such a "block" of tumors may serve as a more realistic predictive test-system for general clinical activity than the response of any one tumor. Studies with the 13762 mammary adenocarcinoma model, predictive for those certain human tumors susceptible to PAM and CTX, show (1) a drug synergism when either is combined with 5-FU and MTX; (2) a marked enhancement of the remission induction and maintenance activity of PAM when tumors were pretreated with ADR in a drug crossover sequence; (3) that after induction of maximum remission, continuing PAM treatment retards the growth of PAM resistant tumors, an inhibitory effect which persists for about 2 weeks if treatments are discontinued; (4) that 5-FU added to a PAM regimen at maximum remission prolonged remission maintenance.

Significance for Cancer Research (NCP Objective 6C Approach 1)
Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Treatment Site Visit Date: 3/22/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $157,000

CONTRACT RESEARCH SUMMARY

Title: Surgical Adjuvant Chemotherapy in Patients with Breast Cancer

Principal Investigator: David L. Ahmann, M.D.
Name/Address Mayo Clinic
Performing Organization: Rochester, Minnesota 55901

Contract Number: NO1-CB-33899
Starting Date: 6/30/75 Expiration Date: 6/29/77

Goal: To assess the effects of surgical adjuvant chemotherapy utilizing a multiple drug program, cyclophosphamide, 5-fluorouracil and prednisone (CFP), compared to CFP plus irradiation therapy and a program with phenylalanine mustard alone.

Approach: Patients with unfavorable primary breast cancers have been assigned to treatment programs involving mastectomy followed by multiple drug chemotherapy (CFP), multiple drug chemotherapy (CFP) with postoperative irradiation and single agent chemotherapy utilizing phenylalanine mustard. After stratification according to menstrual category, tumor size and degree of nodal involvement, each patient will be randomly assigned to one of the three treatment programs as indicated above. The adjuvant chemotherapy will be initiated two weeks postoperatively and will be given concomitantly with radiation therapy when this modality also is employed. Chemotherapy is to be terminated at the end of one year (10 courses) or upon the appearance of metastatic disease, whichever should occur first.

Progress: Patient entry in the study began in September 1973. As of January 1976, a total of 90 patients had been entered on study, 72 thus far evaluable. There have been three recurrences in patients receiving a former treatment which involved radical mastectomy alone and which was subsequently abandoned. There have been no recurrences in the phenylalanine mustard treatment group thus far; the interval of disease-free periods varies from 10 to 47 weeks. Combination chemotherapy program CFP has been associated with recurrences in 6 of 22 evaluable patients, 3 recurrences became evident following cessation of chemotherapy. Of 24 evaluable patients who received CFP and radiation therapy, only one has shown recurrent cancer thus far and this was at 60 weeks following completion of the chemotherapeutic program. There have been no drug deaths. Myelosuppression is a usual accompaniment to the cytotoxic chemotherapy programs; it seems to be least severe with phenylalanine mustard and most pronounced with the addition of radiation therapy of the CFP program. Continued statistical analysis is an ongoing part of this project.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment Site Visit Date: Pending
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $100,000

CONTRACT RESEARCH SUMMARY

Title: Suppression of Endocrine Function by Systemic Agents for Breast Cancer Therapy

Principal Investigator: Richard J. Santen, M.D.
Name/Address The Milton S. Hershey Medical Center
Performing Organization: of the Pennsylvania State University
Hershey, Pennsylvania 17033

Contract Number: NO1-CB-53851
Starting Date: 5/15/75 Expiration Date: 5/14/77

Goal: To produce suppression of adrenal function with aminoglutethimide and to compare the effects upon human breast cancer with those of adrenalectomy.

Approach: Female patients with inoperable, recurrent, or metastatic breast cancer are admitted to this study which is expected to establish the optimal technique for aminoglutethimide (AG) blockage of adrenal steroid synthesis and glucocorticoid suppression of the reflex rise in ACTH. The feasibility of suppressing ovarian function and AG and a progestin will be investigated. The influence of AG upon prolactin secretion will be studied *in vivo* and *in vitro*. The effects on human breast cancer of endocrine suppression by these non-invasive techniques will be compared with those of surgical ablation.

Progress: Endocrine kinetic studies revealed that aminoglutethimide does not interfere with the metabolism of hydrocortisone in contrast to dexamethasone. A regimen consisting of aminoglutethimide in conjunction with hydrocortisone is preferable to that using dexamethasone with respect to uniformity of adrenal suppression. Early studies of medical oophorectomy with aminoglutethimide are promising.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., E. Brad Thompson, M.D.
Program: Breast Cancer Treatment Site Visit Date: 3/7/75
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $220,000

CONTRACT RESEARCH SUMMARY

Title: Cell Kinetics of Breast Cancer

Principal Investigator: Joseph Post, M.D.
Name/Address Goldwater Memorial Hospital
Performing Organization: New York University
New York, New York 10017

Contract Number: NOI-CB-33903
Starting Date: 6/28/73 Expiration Date: 6/27/77

Goal: To provide information concerning cell cycle parameters in patients with breast cancer.

Approach: A. In Vivo: 1) ^{3}HTdR is given i.v. as a pulse label 7.5-10 mCi/pt and autoradiographs of serial biopsies are made to develop a labeled mitosis curve and to record grain count decay; 2) ^{3}HTdR is given as continuous label, 5 mCi/24 hours over several days and lesions are biopsied serially. These techniques permit estimation of population engaged in DNA synthesis, cell cycle parameters and the source of new cells. B. In Vitro: 1) Enzymatically dissociated cells from biopsies and primary surgical specimens are studied by pulse and continuous labeling, along with double-labeling (^{3}H and ^{14}CTdR) for S times to characterize the proliferative patterns of breast cancer cells. 2) Parallel kinetic studies are made on cells cultured from specimens in A, as well as from primary surgical specimens, as outlined in A. In addition, chromosome estimates are made on colcemid collected mitoses.

Progress: 1) In vivo data (8 pts.) show small proliferating pools (<10%). PLM curve reveals T_{G2} + M/2 of 4 hours and T_S of 24 hours with no second wave after 139 hours. There is a wide range of intermitotic times, many cells spending long intervals in G_1 and in G_2. The PLM curve is composed chiefly of the "fast replicators." 2) In 44 patients, collagenase-dissociated cells labeled in vitro had labeling indices and T_S similar to cells labeled in vivo. Such cytokinetic data provide a basis for planning specific drug therapy directed toward proliferating and resting cells, as well as its monitoring. In addition, the data may be related to the clinical course. 3) Log phase cultured cells (from 13 pts.) have larger proliferating pools and faster replication than cells in 1) and 2). Chromosome estimates (3) show wide ranges of chromosome numbers, up to 120 per cell.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: Mary E. Sears, M.D., Stanley Shackney, M.D.
Program: Breast Cancer Treatment Site Visit Date: 10/31/74
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $120,000

CONTRACT RESEARCH SUMMARY

Title: New Techniques in Cell Kinetics of Breast Cancer

Principal Investigator: Robert M. Zucker, Ph.D.
Name/Address Papanicolaou Cancer Research Institute
Performing Organization: Miami, Florida 33123

Contract Number: NO1-CB-33861
Starting Date: 6/27/73 Expiration Date: 2/26/77

Goal: Develop an approach to cell kinetics which is potentially adaptable to the study of human breast cancer kinetics and the cellular effects of chemotherapy.

Approach: The project utilizes a series of differing morphological animal tumors to acquire information on the procedures necessary to obtain a single cell suspension from the solid tumors. Using a combination of enzymatic and mechanical agitation, viable cells are obtained from the tumors that will be used for cell separation and in vitro tissue culture experiments. The dissociated cells will be characterized by kinetic, morphological, cell separation, and electronic cell volume analysis data. The effects of chemotherapeutic agents administered singly and in combination to tumor-bearing animals will be studied on the dissociated and physically separated cells. The in vitro dissociation procedures developed on animals are now being applied to human breast tumors.

Progress: Four different types of rat and mouse model tumor systems have been characterized by light and electron microscopy and then optimally dissociated using a combination of enzymatic and mechanical methods. The factors responsible for the dissociation have been investigated to yield the basis of broad applications to human and other animal tumors. Over 80% viable cells consistently have been obtained with the four tumors used. Studies using in vitro and in vivo H^3 thymidine labeling combined with the cell separation procedures of velocity sedimentation and buoyant density gradient centrifugation have been made with the dissociated cells. Using this methodology the comparative effects of PAM + 5-FU injected singly and in combination were studied on the 13762 mammary adenocarcinoma and MeCCNU, cyclophosphamide and Palmo Ara-C were studied on the B_{16} melanoma. Different interactions between the tumor and the drugs were revealed by morphological and biophysical parameters.

In addition to morphological and autoradiographic analysis the dissociated and separated tumor cells were characterized by electronic volume analysis and fluorescent mithramycin flow analysis. It was determined that the B_{16} had a normal chromosomal content while the 13762 chromosome had approximately 1.5 times the normal content.

Significance for Cancer Research (NCP Objective 5 Approach 6)
Project Officer: Mary E. Sears, M.D., Stanley Shackney, M.D.
Program: Breast Cancer Treatment Site Visit Date: 10/31/74
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $116,000

CONTRACT RESEARCH SUMMARY

Title: Steroid Sulfation and Estrogen Binding in Human Breast Cancer

Principal Investigator: Thomas L. Dao, M.D.
Name/Address Roswell Park Memorial Institute
Performing Organization: Buffalo, New York 14263

Contract Number: N01-CB-43900
Starting Date: 2/1/74 Expiration Date: 1/31/77

Goal: To determine the relationship of steroid sulfotransferase activity to estrogen receptor protein levels, to pathologic staging, and to risk of relapse.

Approach: Steroid sulfating enzyme activity and estrogen receptor (ER) levels will be assayed on breast tissue obtained from approximately 300 patients in three institutions. The evidence will be evaluated for or against correlation of the steroid sulfotransferase activity with risk for tumor metastasizing potential and with histopathological parameters. The predictive potentials of ER and sulfation activity will be compared.

Progress: A total of 203 patients have been entered into the study. Composite data from three cooperating institutions suggest that breast tumors from patients with no axillary metastases are actively synthesizing DHEA sulfate while the number of tumors lacking ERP is largest in patients without axillary nodal metastasis. Of 76 tumors from patients with no axillary nodal metastasis, 32 (43%) are inactive for ERP. Only 10 out of 44 tumors from patients with axillary node metastases are inactive (23%). The ratio of DHEAS to E_2S appears to be independent of the degree of nodal metastasis. The time of follow-up is still too short for meaningful analysis of the relationship between these biochemical parameters and the recurrence rate.

Significance for Cancer Research (NCP Objective 6 Approach 6,4)

Project Officer: Mary E. Sears, M.D., D. Jane Taylor, Ph.D.
Program: Breast Cancer Treatment Site Visit Date: 6/5/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $97,000

CONTRACT RESEARCH SUMMARY

Title: Hormone Manipulation Plus Chemotherapy in Breast Cancer Patients

Principal Investigator: Charles Perlia, M.D.
Name/Address Rush-Presbyterian-St. Luke's Medical Center
Performing Organization: Chicago, Illinois 60612

Contract Number: NO1-CB-43991
Starting Date: 6/17/74 Expiration Date: 6/16/77

Goal: To assess the effect of combination chemotherapy-oophorectomy for the treatment of metastatic breast cancer.

Approach: Premenopausal patients undergo surgical castration upon demonstration of the first relapse following treatment of primary breast cancer. When evaluation is carried out 12 weeks after castration, anti-tumor effect is classified as failure, no change, or regression. The patients showing no change or tumor regression are randomly assigned to 1) observation or 2) treatment courses or cyclophosphamide, 5-fluorouracil, and methotrexate (CMF). When relapse is documented in the patients assigned to observation, CMF treatment will be started. This program provides comparison of the effectiveness of concomitant and sequential multimodal therapy.

Progress: The collaborating investigators began entry of patients into the study in August 1974. More than 50 patients have been registered but only 15 have been randomized post castration, with 9 being randomized into combination chemotherapy and 6 for observation. As of this date, there has been insufficient accrual and time to produce conclusive data.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment Site Visit Date: Pending
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: None (Time extension only)

CONTRACT RESEARCH SUMMARY

Title: Prolactin Radioimmunoassays in Human Serum

Principal Investigator: W. P. VanderLaan, M.D.
Name/Address Scripps Clinic and Research Foundation
Performing Organization: La Jolla, California 92037

Contract Number: NO1-CB-33881
Starting Date: 6/29/73 Expiration Date: 3/28/76

Goal: To perform radioimmunoassays of prolactin in serum from women who have breast cancer.

Approach: Improvement of the hPRL-RIA has been a main concern. Antibody production with highly purified hPRL as antigen has been achieved, although not in the original high titer of the first antibody. The appropriate handling of PRL has been studied in terms of storage and proper conditions for iodination. We have studied the nature of the iodination products and their effects on the binding of iodinated prolactin to antibody. Other factors in the standardization of the assay will, in the main, have been studied before the contract expires.

Progress: Although the prolactin radioimmunoassay remains technically rather difficult at present, substantial improvement has been achieved. In addition, we have made available, through the National Pituitary Agency, to the Hormone Distribution Program, materials for human prolactin radioimmunoassays. Here the demand has been enormous. We had an urgent request for more antibody at a time when we had already provided sufficient antibody for 20,000,000 radioimmunoassay tubes to have been set up. We have also provided technical advice to many investigators, and these factors probably have contributed to the sharp decline in the number of specimens submitted for assay at these laboratories.

In addition to the hPRL-RIA,,we have initiated measurement of a naturally occurring cleaved form of human growth hormone assaying 15-18 IU/mg in the pigeon crop sac as well as 5-7 IU/mg in the hypophysectomized rat tibial or body weight gain test. Measurement is being attempted both by radioimmunoassay and radioreceptor techniques. (Reference: Lewis, U.J., Singh, R.N.P., Peterson, S.M. and VanderLaan, W.P., Submitted to the Third International Symposium on Growth Hormone and Related Peptides, Milan, September 1975).

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Mary E. Sears, M.D., D. Jane Taylor, Ph.D.
Program: Breast Cancer Treatment Site Visit Date: 4/2/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: None; Terminated

CONTRACT RESEARCH SUMMARY

Title: Therapy of Patients with Stage II or Stage III Carcinoma of the Breast

Principal Investigator: Frank C. Sparks, M.D.
Name/Address University of California, Los Angeles
Performing Organization: Los Angeles, California 90024

Contract Number: NO1-CB-43917
Starting Date: 6/17/74 Expiration Date: 6/16/77

Goal: To evaluate the effectiveness of adding chemotherapy and immunostimulation to mastectomy for patients with locally advanced cancer.

Approach: Patients with any number of positive axillary lymph nodes are eligible to enter this study, which randomly assigns one of the following treatment schedules: 1) cyclophosphamide, methotrexate, 5-fluorouracil (CMF) 2) CMF plus BCG or 3) CMF plus BCG plus tumor cell vaccine. The tumor vaccine is produced from irradiated cells. Treatment will be given intermittently for a period of 2 years, or until there is evidence of tumor recurrence, whichever occurs first. The patients undergo evaluation of immunologic parameters before treatment begins and at intervals throughout the study. The effect of live tumor cell vaccine is being tested in a group of women with metastatic breast cancer. If no evidence of local tumor proliferation occurs, the live vaccine will be considered for adjuvant use.

Progress: None of the 50 patients entered to date has recurrence, with an average follow-up period of 30 weeks (range 12 to 70) after mastectomy. The clinically disease free status of the patients who had >4 metastatic nodes is particularly encouraging. Toxicity has been acceptable. Immunocompetence, as measured by delayed cutaneous hypersensitivity to PPD and DNCB, has been maintained.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment Site Visit Date: 10/10/75
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $200,000

CONTRACT RESEARCH SUMMARY

Title: Prediction of Hormone Dependency in Human Breast Cancer

Principal Investigator: Elwood V. Jensen, Ph.D.
Name/Address University of Chicago
Performing Organization: Chicago, Illinois 60637

Contract Number: NO1-CB-43969
Starting Date: 6/16/66 Expiration Date: 6/15/77

Goal: To develop techniques which will increase the predictability of response of human breast cancers to endocrine therapy.

Approach: Since 1966 Dr. Jensen has been studying estrogen receptors (ER) in human breast cancer tissues and the relationship between ER content and response to endocrine manipulative treatments. Patients who have had ER assays performed on their primary and/or metastatic tumors undergo additive or ablative endocrine procedures considered appropriate for their gonadal status, general medical condition, etc. In most instances the choice of therapy does not depend on the ER assay results. The data are analyzed for correlation between clinical response and ER levels. The investigators are also measuring the binding of a progestational agent (Roussel RC-5020) by the same tumor specimens, and they are working on improvements of the estrophile assay method, in particular a simple radioimmunoassay method.

Progress: 705 primary breast tumors have been studied, and about 30% found to contain significant amounts of ER (>750 femtomoles/gram tissue in postmenopausal patients and >250 femtomoles in premenopausal). These patients are being followed for recurrence of disease and response to endocrine therapy at that time. Of the patients with metastatic breast cancer, 133 underwent some type of endocrine therapy, the clinical results of which could be evaluated. Forty-six of these patients had ER positive tumors and 29 (63%) showed objective remissions. Only 2 of the 87 (3%) patients whose tumors showed less than significant amounts of ER had objective tumor remissions. Preliminary observations with 13 patients suggest that ER determinations in the primary tumor at the time of mastectomy may be used to predict tumor response to endocrine therapy if metastases appear in later years. Attempts to develop a simple radioimmunoassay for estrophilin are in their initial stages. Rabbits have been immunized with a highly purified preparation of the nuclear estradiol-receptor complex of calf uterus. As soon as sera are obtained that can be shown to contain antibodies, cross reactivity with the receptor of human breast cancer will be tested. Meanwhile purification of receptor from human uterus is being undertaken on a large scale to provide human estrophilin for antibody preparation.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: Mary E. Sears, M.D., Ihor Masnyk, Ph.D.
Program: Breast Cancer Treatment Site Visit Date: 10/23/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $110,000

CONTRACT RESEARCH SUMMARY

Title: Endocrine Therapy Plus Chemotherapy in Patients with Breast Cancer

Principal Investigator: David T. Kiang, M.D., Ph.D.
Name/Address University of Minnesota School of Medicine
Performing Organization: Minneapolis, Minnesota 55455

Contract Number:
Starting Date: Expiration Date:

Goal: To study the effect on metastatic breast cancer of combining chemotherapy with estrogen therapy and to assess the value of estrogen receptor assays for treatment selection.

Approach: Patients eligible to enter this study program will be women who are showing the first evidence of metastatic breast cancer. Estrogen receptor (ER) assay results will determine the pair of treatment regimens to be randomly assigned after stratification according to disease free interval and disease dominant site. The patients with ER negative cancer, will receive 1) Diethylstibestrol (DES) plus cytoxan and 5-fluorouracil (CF) or 2) CF alone. Those with ER positive cancers will receive either 1) DES and CF in combination or 2) DES alone followed by CF with evidence of DES treatment failure or of tumor progression after initial regression. The patient on whom ER assays cannot be obtained will have the same treatment assignments as those with ER positive tumors.

The incidence and duration of remissions and survival data will be analyzed to determine 1) the effect of combining standard hormonal therapy and chemotherapy and 2) the value of ER assay results in predicting the clinical response to therapy.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D.
Program: Breast Cancer Treatment Site Visit Date:
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $95,000

CONTRACT RESEARCH SUMMARY

Title: Primary Breast Cancer Group (NSABP)

Principal Investigator: Bernard Fisher, M.D.
Name/Address University of Pittsburgh
Performing Organization: Pittsburgh, Pennsylvania 15261

Contract Number: NO1-CB-23876
Starting Date: 6/30/72 Expiration Date: 6/29/77

Goal: To determine the effectiveness of local treatments for Stage I breast cancer and of local plus systemic treatments for Stage II breast cancer.

Approach: The investigations are prospective and randomly assign a treatment program to each patient. Investigators from approximately 88 collaborating institutions participate. Patients with clinical Stage I breast cancer were entered into the study which tests the relative merits of two or more currently accepted forms of surgical and/or radiation treatments. Morbidity and cosmesis are considered as well as relapse rates and survival. Patient entry was completed and follow-up is being carried out. In a separate clinical trial, patients with one or more positive axillary nodes receive l-phenylalanine mustard (l-PAM) or l-PAM plus 5-fluorouracil (5-FU) in an intermittent dosage schedule for two years or until evidence of treatment failure is documented, whichever comes first. The chemotherapy begins 2 to 4 weeks after mastectomy.

Progress: The NSABP investigators completed entry of patients into two large studies and reported their preliminary results. In 1665 patients who had undergone either radical mastectomy, or total (simple) mastectomy or total mastectomy plus radiotherapy, no significant differences have occurred in treatment failure rates or survival. These patients will be observed indefinitely to determine long-term effects. In the other completed study patients who had demonstrated tumor metastases in axillary nodes at the time of mastectomy received long-term intermittent l-PAM or placebo pills. The early tumor recurrence rate is significantly lower in the premenopausal patients who received active drug and who has positive nodes. As of December 1975, postmenopausal patients who received active drug also demonstrated improvement that was significant at the 0.05 level.

Presently, 400 patients have been entered on study to evaluate l-PAM vs. l-PAM + 5-FU. It is too soon to report data on that study.

A new protocol relative to segmental mastectomy has been activated and at least two new adjuvant chemotherapy studies are almost ready for implementation.

Significance for Cancer Research (NCP Objective 6 Approach 1)
Project Officer: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment Site Visit Date: 12/14/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $520,000

CONTRACT RESEARCH SUMMARY

Title: Estrophile Binding and Estrophile Proteins in Human Breast Cancer

Principal Investigator: Federico Welsch, M.D., Ph.D.
Name/Address Worcester Foundation for Experimental Biology
Performing Organization: Shrewsbury, Massachusetts 01545

Contract Number: NO1-CB-43867
Starting Date: 10/16/73 Expiration Date: 10/15/76

Goal: To determine whether the levels and properties of estrophilic proteins are related to the responsiveness of cancer to endocrine treatment.

Approach: This is a type I contract in that the contractor carries out determinations of estrophilic receptor protein (ER) levels on 500 breast cancer samples per year. These tissues are submitted by collaborating clinicians whose protocols have been approved by the project officers. The ER assays are performed by both the sucrose gradient and the charcoal methods and methodology is under constant monitoring. The laboratory also performs steroid sulfurylation determinations on tumor samples of sufficient size. The project officers receive the reports of the ER assay results from the laboratory and are also provided with clinical data by the collaborating physicians. The combined data will be analyzed after accession of about 1000 tissues to determine the degree of correlation between ER levels and tumor response to treatment.

Progress: The contractor has reached the capacity of 500 assays per year and applications have exceeded that number. The new protocols are being evaluated for relevance to the program. If it appears that more than 500 suitable tissues are available, additional support will be sought.

Significance for Cancer Research (NCP Objective 5B Approach 6)

Project Officer: Mary E. Sears, M.D., D. Jane Taylor, Ph.D.
Program: Breast Cancer Treatment Site Visit Date: 6/7/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 76 Funds: $92,000

ANNUAL REPORT SUMMARY

CANCER DIAGNOSIS CONTRACT PROGRAM
July 1, 1975 to June 30, 1976

The Diagnosis Branch of the Division of Cancer Biology and Diagnosis has expanded its contract research effort rapidly since the Branch was reactivated in 1972. Its current research program, which is operated entirely by contracts, encompasses the categories listed below in the table. Expert advice for the program is provided by the advisory committees as indicated.

Research Categories	Advisory Committees
General Cancer Diagnosis	Diagnostic Research Advisory Group
Breast Cancer Demonstration Projects	Breast Cancer Diagnosis Committee
Breast Cancer Diagnosis	
Diagnostic Radiology	Diagnostic Radiology Committee
Cytology Automation	Committee on Cytology Automation
Immunodiagnosis	Committee on Cancer Immunodiagnosis

The programs for Breast Cancer Diagnosis, Cytology Automation and Immunodiagnosis are presented in separate sections of this Annual Report; the other categories are described below.

GENERAL CANCER DIAGNOSIS STUDIES

Lung Cancer received early attention in the Diagnosis program. A joint study currently is in progress by a Cooperative Early Lung Cancer Group. In this study each of three projects (at Mayo Foundation, Johns Hopkins University Medical School and Memorial Hospital-Strang Clinic in New York City) will screen at least 5,000 persons who are heavy smokers annually for lung cancer with frequent sputum cytology and chest x-ray examinations. In each project 5,000 other screenees (of equal risk) will be randomly selected to act as a control cohort. Localization of lesions is accomplished with fiberoptic bronchoscopy. The goal is to detect lung cancer at a sufficiently early stage to permit its removal and cure by a surgical procedure that will be less extensive than a pneumonectomy. The University of Cincinnati Statistical Center coordinates accumulation of data from the three cooperating projects. A Manual of Procedures has been compiled by the group. Ten thousand persons have already been screened at the Mayo Clinic, approximately 8,125 persons at the Johns Hopkins Medical School and 6,600 at Memorial Hospital. At Johns Hopkins University Medical School studies continue on cytogenetics of lung cancer cells in sputum, immunological procedures in the detection of lung cancer and other procedures. The Mayo Clinic is studying the role of aryl hydrocarbon hydroxylase enzyme in heavy smokers and persons with lung cancer. At Memorial Hospital in New York City, studies toward the development of a squamous cell antigen related to lung cancer will be undertaken.

Three cooperating institutions, Mayo Foundation, the University of Chicago and Memorial Hospital-Sloan Kettering Institute, are studying, in a predetermined manner by a common protocol, patients who are suspected of having cancer of the pancreas and are about to undergo abdominal surgery. Special studies delineated in the protocol include preoperative and operative study of CEA in peripheral and portal venous blood. Special studies are being made of pancreatic excretions obtained through a fiberoptic scope in the duodenum, pancreatic and biliary ducts. Such secretions are being studied for tumor-associated antigens, abnormal enzyme content, biochemical alterations, as well as cytological changes which will permit the earlier diagnosis of pancreatic cancer. Other diagnostic methods being investigated include imaging with ultrasound, CTT scanning, radionuclides, and retrograde pancreatoduodenography.

Several ongoing projects are concerned with various aspects of diagnosis of gastrointestinal cancer. Studies at University of Rochester and at eight other institutions under the auspices of the American College of Radiology are exploring methods of bowel preparation prior to barium enemas or colonoscopy. At the Mayo Clinic, there is a study of carcinoembryonic antigen in conjunction with other tests for cancer to determine if any of these combinations will assist in the earlier diagnosis of gastrointestinal cancer. Two projects, at Mt. Sinai Medical School and the University of Louisville, have been investigating development of a quick, sensitive method for the detection of human blood in stool specimens. A study at University of Minnesota will investigate the use of a screening technique for human blood in the stool as a means of detecting early bowel cancer and thereby possibly prolonging lives.

Two projects, one at Stanford University and one a joint effort at SUNY-Buffalo and Roswell Park Memorial Institute, are studying biochemical markers which may be of help either in specific or in general cancer diagnosis. Hormone markers for the same purpose are being studied at Harbor General Hospital.

Three projects, at Baylor University College of Medicine, Downstate Medical Center-SUNY and at Johns Hopkins University, have been studying nuclear magnetic resonance in normal, non-neoplastic diseased tissue and neoplastic tissue to determine possible application of NMR to early cancer detection.

One project to evaluate the "Xonics" process for electron radiography is being carried out at Hahnemann Medical College.

New studies were begun during 1975 at Boston University and at Michigan Cancer Foundation to investigate the optimum frequency of screening for early cancer detection. Other new studies, at University of Tennessee and University of Minnesota, will investigate the impact of multisite screening on total cancer mortality.

Development and testing of systems to improve x-ray imaging is being undertaken at University of Alabama and University of Wisconsin in studies begun in 1975.

In addition to the above twenty-seven present projects in General Cancer Diagnosis four others currently are pending. These include one project to carry out periodic screening in relatives of patients with medullary carcinoma, two others to attempt the development of an immunodiagnostic method for early detection of ovarian cancer in asymptomatic women and one project to develop a new serum isoenzyme test for cancer. Contracts are pending for all four projects.

BREAST CANCER DEMONSTRATION PROJECTS

Much of the initial thrust of the Contract Diagnosis program was directed toward research in earlier detection of breast cancer; these efforts culminated in establishment of 27 regional Breast Cancer Demonstration Projects. All 27 of these projects currently are in operation and by spring 1976 approximately 250,000 women had been screened. None of the projects have experienced any serious problems in recruiting which has been carried out in an excellent manner by local American Cancer Society units. Currently, it appears that response to annual recalls may be approximately 90 percent. Recall mechanisms are under constant review. Also under review are methods of physical examination, teaching of breast self-examination, and methods for mammography and thermography reading.

Meetings have been held with the Project Pathologists to define their role in formalizing information obtained from biopsy and other surgical specimens. The Project Pathologists are responsible for completing pathology forms which have been revised and distributed to all projects. The surgical material will be reviewed locally, regionally and centrally; parts of the latter two reviews will be done by sampling. From this effort it is expected that attention will be directed to the pathology of early breast cancers and possibly to its precursors.

Annual meetings of Project Directors and Coordinators are held to aid in standardizing the studies and resolving problems. At the annual meeting in 1975 a thermography workshop was included.

Collection and statistical management of all data from the 27 centers is coordinated at the University City Science Center in Philadelphia. Standard reporting procedures have been developed. Preliminary analysis of data available at the end of 1975 indicates that biopsies have been recommended for approximately 4 percent of those examined. At least one-half of those biopsied have been reported as cancer. These fragmentary results are regarded as quite preliminary and should not be used as a basis for any conclusions.

DIAGNOSTIC RADIOLOGY

In this area of research the improvement of x-ray imaging has been a matter of major concern and one to which the Diagnostic Radiology Committee has devoted much time. For early cancer detection and for screening programs involving periodic recurrent x-rays of individuals, it is extremely important to achieve improved resolution with substantially lower levels of radiation.

Five institutions: Massachusetts General Hospital, Columbia Presbyterian Medical Center, the Mayo Clinic, George Washington University Medical School and the Cornell University Medical School, have projects to perform clinical evaluation of equipment for computer assisted tomography of the brain, a noninvasive technique believed to be of great promise in the field of neuro-radiologic diagnoses. It is anticipated that this new technique will complement and in some instances eventually replace present techniques used in diagnosis of intracranial lesions. Projects to investigate the use of proton beams in tissue density measurement are in progress at the University of California and University of Chicago.

In addition to the above seven projects, all of which were begun in June 1974, seven additional projects were begun in June 1975. All seven are concerned with various aspects of the problem of development of new cancer diagnostic capabilities using computerized transaxial tomography (CTT). One contract, with Mayo Foundation concerns accumulation of a transaxial x-ray projection data base for use in testing algorithms; another with Research Foundation of SUNY is to support development of algorithms for computerized transaxial x-ray reconstruction. Three contracts, with ERDA-Lawrence Berkeley Laboratory, University of Pennsylvania, and Massachusetts General Hospital, involve development of algorithms for computerized transaxial nuclide reconstructions. Two contracts, one with American Science and Engineering and one with Presbyterian Hospital (NY), are supporting development and evaluation of a CTT body scanner.

Several new contracts currently are being negotiated in the general area of diagnostic radiology and work will begin on them within the next few months. These include: (1) work on development and testing of a standard protocol for evaluation of imaging techniques in cancer diagnosis; (2) development of radioisotopic surface markers and detectors for use in endoscopic techniques; and (3) development of specific immunoglobulins labelled with gamma-emitting radioisotopes for external detection of tumors. One or more Cancer Research Emphasis Grants may also be awarded to study methodology for performing mass radiomammography with less than 150 mR per exposure.

CONTRACT RESEARCH SUMMARY

Title: Methods of Bowel Preparation Preparatory to Barium Enema or Colonoscopy

Principal Investigator: Arthur J. Present, M.D.
Name/Address American College of Radiology
Performing Organization: 20 North Wacker Drive
Chicago, Illinois 60606

Contract Number: NO1-CB-43974
Starting Date: 6/15/74 Expiration Date: 6/14/76

Goal: To perfect methods for insuring a clean colon for the benefit of the radiologist and/or the endoscopist. The technique should not require long periods of time (within a 24-hour period) and without excessive dietary restrictions and without excessive patient discomfort or inconvenience.

Approach: Under the administrative supervision of the American College of Radiology, the comparative efficiency of 8 selected colon cleansing regimes will be studied by 7 institutions. A total of 1,400+ patients (200 patients in each institution) will be examined; all regimes will be employed by each of the participating institutions on a randomized basis, standard forms for the collection of pertinent clinical information and for the evaluation of the efficiency of each regime have been prepared and will be completed at each institution. Methods for cross evaluations are also included. Statistical analyses will be performed by the Department of Biomathematics at M.D. Anderson Hospital.

Progress: The project for Development of Methods of Bowel Preparation Preparatory to Barium Enema or Colonoscopy is continuing with the participation of 7 centers. Each of the centers is responsible for preparing 25 patients each by 12 specified protocols. Projects due to protocol contraindications and patient loads presently range from one institution on the second of 12 protocols under study to one institution which has completed all 12. Other centers are either approaching or are past the halfway point within the series. Evaluation is being conducted on protocols by comparison which based on current data indicates two of the 12 specified protocols are superior to the others and also evaluation is being conducted on readers by way of comparison of variances between readers and frequency of rating patterns.

It is anticipated all participants will conclude patient preparations during the extension period currently under request.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg and R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $122,806

CONTRACT RESEARCH SUMMARY

Title: Nuclear Magnetic Resonance To Be Studied in Neoplastic and Non-neoplastic Tissues

Principal Investigator: Carlton F. Hazlewood, Ph.D.
Name/Address Baylor College of Medicine
Performing Organization: Houston, Texas 77030

Contract Number: NO1-CB-43978
Starting Date: 6/30/74 Expiration Date: 6/30/76

Goal: The use of nuclear magnetic resonance spectroscopy for the identification of malignant, neoplastic tissue so that this tissue can be differentiated from normal and non-neoplastic, diseased tissue.
Approach: To determine T_1, T_2 and T_1/T_2 in breast lesions. To conduct a blind study on surgical specimens in conjunction with Department of Pathology. To explore the relaxation time in the rotating frame $T_{1\rho}$, compound relaxation times, temperature dependence of relaxation times, feasibility of using relaxation time in detection of cancer surrounded by normal tissue.
Progress: Using NMR techniques to measure relaxation times T_1 and T_2 of water hydrogen protons, it has been demonstrated that specific morphological states of various tissues can be recognized. In a blind study of over 600 human breast biopsy samples, we were able to show discrimination between normal and malignant tissues on the basis of T_2 but not on T_1. Serial section analysis by pathologists has shown that the samples are very heterogeneous with a mean of less than 20% cancer cells and a mean of over 20% fat in biopsies diagnosed as malignant. At present, we are attempting to surmount this problem with multiple fraction analysis by computer program and the use of low temperatures to decrease the contribution from fat. Statistical analysis of the data has indicated the possibility of a non-Gaussian distribution of NMR values that might lead to a definition of a high risk population in the fibrocystic category.

Additional studies of human breast cancer cell lines grown in tissue culture have shown a possible correlation between the T_1 of the cells and their rate of division or malignancy index. Analysis of the HeLa cell cycle by NMR has revealed a reproducible phase-specific pattern of changes in T_1 and T_2 during the cell cycle. By working with cells, we will be able to understand better the underlying mechanism of difference between normal and malignant tissues.

A study of mouse serum from normal animals and those carrying mammary tumors has shown a very definitive separation of the T_1 values of normal, preneoplastic, and malignant serums. In humans, the study is under way and has so far shown a significant difference in the means of a selected normal control group and a sampling of serums from patients with diagnosed mammary carcinoma.

Significance for Cancer Research (NCP Objective 5 Approach 5)
Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $80,536

CONTRACT RESEARCH SUMMARY

Title: Determination of Optimal Frequency of Screening Strategies

Principal Investigator: Arthur Albert, Ph.D.
Name/Address Boston University
Performing Organization: Boston, Massachusetts 02215

Contract Number: N01-CB-53863
Starting Date: 6/1/75 Expiration Date: 5/31/77

Goal: To develop a means whereby the optimum frequency of screening can be determined for early cancer for various anatomic sites in order to assure success in such screening as the least possible cost and hazard to the patient and still be effective in reducing death from cancer.

Approach: A team of clinicians, pathologists, statisticians, operations researchers and epidemiologists will carry out extensive investigation and documentation of the level of present knowledge, both empirical and theoretical on the following five factors: 1) the natural history or development of the disease, 2) the incidence of the disease in the target population, 3) the effectiveness (sensitivity and specificity) of the various available screening tests at each stage of the disease, 4) the effectiveness (on mortality) of intervention (treatment during each stage of the disease) and 5) the cost and hazards of the various screening tests. Disease models will be developed and validated for which screening data exists, or must be developed, such as colon, lung, bladder and prostatic cancer. Through parametric analysis these models will be used to suggest general ranges or mimimum limits on the performance of screening tests if they are to be effective in early cancer detection and reducing cancer mortality.

Progress by March 1, 1976: Have developed general model for the natural history of progressive chronic disease. Have developed methodology for describing the impact of screening and dynamic demographic factors on the natural history of disease in residual population. Have begun to develop cost models. Have begun to develop procedures for collecting and analyzing data to determine natural history parameters. Have begun to program simulation studies. Have begun to identify potential sources of data already in existence.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $113,000

CONTRACT RESEARCH SUMMARY

Title: Clinical Application and Evaluation of "Xonics" Electron Radiography

Principal Investigators: Luther W. Brady, M.D. and Leonard Stanton, M.S.
Name/Address Hahnemann Medical College and Hospital
Performing Organization: Philadelphia, Pennsylvania 19102

Contract Number: NO1-CB-43992
Starting Date: 6/30/74 Expiration Date: 6/29/76

Goal: An evaluation of both present and improved versions of the Xonics x-ray imaging system, in order to report the following information: 1) Basic scientific information for technique design and optimization; 2) Extensive clinical experience to determine the most promising use of electron radiography, as well as studies where conventional radiography may be preferable; and 3) New approaches to the basic problem of comparing the ability of different imaging systems to demonstrate marginally detectable pathology.

Approach: In order to accomplish these objectives, the contractor will make basic scientific measurements as to response to x-rays, detail resolution, noise and reliability and reproducibility of clinical equipment. Finally, a clinical comparison and evaluation will be performed involving 16 nationally recognized diagnostic radiologists who are very interested in contributing the the evaluation process. This will include normals (4%) as well as films demonstrating pathology; pairs will not be presented as pairs, nor will each evaluator necessarily receive pairs.

Progress: Technical studies have provided the basis for an estimate of the ERG system potential. ERG image contrast and edge characteristics depend on the extent of development, particle size, and liquid vs. aerosol deposition. The "Xerox-like" images of the latter can be greatly modified, but so far we have been limited by toner availability to liquid development. This produces sharper than conventional images, showing small details well with modest edge enhancement. In principle, ERG useful sensitivity should be comparable to that of fast screens. Engineering problems remain, notably toner production and control. A unique library of ERG-conventional comparisons now exists. However, the planned definitive evaluation has been prevented by insufficient numbers of suitable comparisons, for two reasons. The first relates to the experimental nature of our exposure and liquid development systems; these are only now becoming dependable. The second is the difficulty of accumulating patients with proven marginally detectable pathology for comparison of detection yields of alternative systems. To solve this problem we have recently developed simulation technology for lytic bone and nodular lung lesions. Examples will be shown in the forthcoming site visit.

Significance for Cancer Research (NPC Objective 5 Approach 3)

Project Officer: Louis P. Greenberg and R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 1/22/74 and 3/26/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $82,311

CONTRACT RESEARCH SUMMARY

Title: Hormone Markers for the Detection and Diagnosis of Cancer

Principal Investigator: William D. Odell, M.D., Ph.D.
Name/Address Harbor General Hospital
Performing Organization: Torrance, Los Angeles, California 90502

Contract Number: NO1-CB-43903
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: To develop hormone markers that might indicate the presence of small (early) treatable cancer.
Approach: 1) To use techniques and assays developed to measure ADH, vasotocin, oxytocin, alpha and beta MSH, ACTH, alpha and beta TSH, and alpha and beta HCG to search for tumor elaboration of these peptides among cancer patients; 2) To develop new techniques for separating and easily identifying "big forms" or "prohormone" forms of ACTH, gastrin, and pancreozymin-cholecystokinin (PZ-CCK); 3) To search for the existence of such big forms of: ADH, oxytocin, alpha and beta MSH, luteinizing hormone, and alpha and beta glycopeptides; 4) To develop peptide assays for fetal hormones related to vasopressin, such as mesotocin as well as other fetal peptides and proteins such as fetuin, and to search for their elaboration by cancers; and 5) To use the insights and techniques gained in these studies to identify other nonhormonal peptides which might be used as cancer markers.
Progress: Our initial comparison of vasopressin, vasotocin, alpha TSH, alpha HCG, alpha and beta MSH, ACTH and parathormone levels in blood samples from patients with many different types of cancer and from a control population of men over fifty, postmenopausal women, men and women under fifty years, did not show any peptide or group of peptides which were elevated above control levels in a significant number of cancer patients. The immunoassayable hormone levels in tumor tissue extracts revealed significant amounts of several hormones. A systematic study was conducted of patients with specific tumor types including lung carcinoma, gastric and esophageal carcinoma, colon and rectal carcinoma pancreatic carcinoma; and also tissue specimens, matched blood samples and bronchial washings collected. Patients are followed with serial blood samples and clinical estimate of tumor mass and activity on therapy. Clinical records will be compared with tumor mass and activity on therapy. Clinical records will be compared with hormone levels in blood in order to examine possible correlation between hormone levels and tumor mass or virulence. We will collect within a year 150 patients with lung or bronchial carcinoma, 100 patients with colon or rectal carcinoma, 50 patients with esophageal and gastic carcinoma and 20-30 patients with pancreatic carcinoma. Patients in each tumor group represent 1/3 of those seen each year by the collaborating institutions. Plasma and tissue samples with elevated hormone levels are being chromatographed and the quantity of differing molecular weight forms measured by immunoassy of the eluate.
Significance for Cancer Research (NCP Objective 5 Approach 4)
Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 2/28/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $153,350

CONTRACT RESEARCH SUMMARY

Title: Lung Cancer Control: Detection and Treatment

Principal Investigator: John K. Frost, M.D.
Name/Address Johns Hopkins University
Performing Organization: Baltimore, Maryland 21218

Contract Number: NO1-CN-45037
Starting Date: 9/1/73 Expiration Date: 8/31/76

Goal: To determine the effect of the early detection of lung cancer by sputum cytology followed by surgical removal on long-term survival in the disease (Phase II of Hopkins Study).

Approach: The study population will be males, 45 years or older, who smoke a pack or more of cigarettes a day. By random assignment from individuals responding to advertisement, it is planned to accumulate over several years two groups of 5,000 each. The test group will receive sputum cytologic examination and chest X-ray every four months; the control group will have an annual chest X-ray, present good medical practice. Subjects suspicious or positive for cancer will be carefully worked up, including fiberoptic bronchoscopy, and cancers will be removed surgically. All subjects will be followed for at least 5 years to determine actuarial survival. (See also NO1-CB-92172).

Progress: There have been 8,125 people screened to date in this program. A return rate of 3% was obtained from the miscellaneous group contacted by mailings; while a return rate of 5% was obtained from the others industry and government. Screening was done at Johns Hopkins Hospital (downtown East Baltimore) and Social Security (Baltimore beltway). Cancers found to date at approximately 6 per 1,000 screenees, 5.4/1,000 in the control X-group and 6.8/1,000 in the cytology C-group. For every 5 cases of lung cancer detected by chest X-ray to date in this project, there have existed an additional 2.06 cases of lung cancer detected only by cytology. Localization of these early lesions has been made by techniques of bronchoscopy and cytopathology which are still being developed in Phase I (NO1-CB-92172) of Hopkins study. This Phase II project is carried on in conjunction with Mayo, Memorial, and Cininnati in the NCI Cooperative Early Lung Cancer Group.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Early cytologic detection, the best present hope for improving lung cancer mortality, requires rigid proof of its effectiveness.

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 6/73 and 2/3/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $700,000

CONTRACT RESEARCH SUMMARY

Title: Lung Cancer - Early Detection, Localization, and Therapy

Principal Investigator: John K. Frost, M.D.
Name/Address Johns Hopkins University
Performing Organization: Baltimore, Maryland 21218

Contract Number: NO1-CB-92172
Starting Date: 6/28/69 Expiration Date: 6/27/76

Goal: 1) Develop improved cytologic methods for sputum examination for the early detection of lung cancer; 2) Develop improved methods for the diagnostic localization of lung cancer (Phase I of Hopkins Study).

Approach: The project follows a group of chemical and asbestos workers at high risk of lung cancer for sputum cytology studies. These are directed to the biologic significance of the morphology of mild, moderate and severe cell atypia, by correlation with patient follow-up. Patients cytologically suspicious or positive for cancer are studied for better localizaton techniques, mainly with tantalum dust bronchography and fiberoptic bronchoscopy. Improved equipment is also under development. (See also NO1-CN-45037)

Progress: Clinical material was obtained from 2,165 cigarette smokers, asbestos and bis-CMME workers. Developed improved transbronchial televison microscope and new methods to diagnose cancer cytologically and histologically from areas of lung heretofore unobtainable. For x-ray negative lesion localization, developed controllable catheter tantalum bronchography and various instruments for rigid and flexible fiberoptic bronchoscopy. Surgical techniques developed for improved therapy of early lesions. Techniques evolved from this Phase I are used in J. H. Phase II, and by Mayo and Memorial in Early Lung Cancer Cooperative Study. Continuing: Investigation of the biologic significance of cytologic high-suspect lesions; parameters for automatic recognition of lung cancer; development of transbronchoscope probes for mucosal tension, bronchial wall firmness, infrared sensing, thermo-recovery, laser, electrocautery, and ultrasonic forceps; development of clinical inhalation for tantalum bronchography.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Early detection is the best present hope for improving the mortality from lung cancer.

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 5/7/74 and 2/3/75
Technical Review Group: Diagnostic Research Advisory Group:
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $350,000

CONTRACT RESEARCH SUMMARY

Title: Nuclear Magnetic Resonance To Be Studied in Neoplastic and Non-neoplastic Tissues

Principal Investigator: Donald P. Hollis, Ph.D.
Name/Address Johns Hopkins University
Performing Organization: Baltimore, Maryland 21218

Contract Number: NO1-CB-43911
Starting Date: 6/1/74 Expiration Date: 5/31/76

Goal: The use of nuclear magnetic resonance spectroscopy for the identification of malignant, neoplastic tissue so that this tissue can be differentiated from normal and non-neoplastic, diseased tissue.
Approach: To carry out measurements of T_1, T_2, and $T_{1\rho}$ on a variety of malignant, normal, and diseased but not malignant tissues removed at surgery; these will be carried out blindly in conjunction with the Department of Pathology. There will also be an evaluation of the sensitivity of NMR measurement to small quantities of malignant cells mixed with normal cells.
Progress: Thus far, primarily T_1 measurements have been made. To date, 279 specimens of human tissue, removed from 118 separate patients in the Johns Hopkins Hospital, have been examined. These specimens included normal tissue, benign neoplastic tissues and various kinds of cancers.
Based on the data gathered to date it is concluded that NMR cannot be used in this direct manner for diagnosis of human cancer because it is not specific for cancer cells. Water content of the tissue is the main determining factor in fixing the value of the T_1 relaxation time. It still is possible that NMR may have a valuable role in cancer detection in the sense of screening from a population those who have a higher than normal likelihood of bearing malignancy. Attempts to estimate the percent of cancer or the percentage of actual malignant cells within the abnormal tissues examined failed to improve the reliability of the attempted diagnosis of cancer by proton magnetic resonance. Efforts to improve the diagnosis by use of partially relaxed spectra rather than null T_1 measurements were not successful, since it proves very difficult to resolve relaxation times whose values are not greatly different. We have concluded that the main impediment to the accurate diagnosis of cancer by proton nuclear magnetic resonance is the fact that the relaxation time is not a specific property of cancer.
Some preliminary results on phosphorus 31 NMR of tissues have been carried out. It has been demonstrated that excellent phosphorus spectra can be obtained on tissues such as liver and kidney within a few minutes using Fourier Transform techniques. Although the phosphorus spectra from different tissues are not identical, we have no data as yet to indicate whether there will be anything special about cancerous tissue as compared to benign tissue.
Significance for Cancer Research (NCP Objective 5 Approach 5)
Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 3/12/76
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis and Steering Committee
FY 76 Funds: $0

CONTRACT RESEARCH SUMMARY

Title: Detection and Localization of Early Lung Cancer

Co-principal Investigators: Robert S. Fontana, M.D.; David R. Sanderson, M.D.
Name/Address
Performing Organization: Mayo Foundation
Rochester, Minnesota 55901

Contract Number: NO1-CB-53886 (Successor to Contract NO1-CB-22000)
Starting Date: 9/27/74 Expiration Date: 9/26/78

Goal: (1) Improve methods for the early diagnosis of lung cancer, (2) Assess survival of patients with lung cancer detected by these methods.

Approach: A population of "high-risk" Mayo Clinic outpatients (i.e., middle-aged and older men who are heavy cigarette smokers) who enter for conditions other than lung cancer are examined and randomized into two groups. A close surveillance group receives chest x-ray examinations and sputum cytology tests three times a year. A standard-surveillance group receives chest x-rays and sputum cytology examinations when first seen and advice to have follow-up chest x-rays and sputum tests annually. Almost all patients suspected of lung cancer either by cytology or by x-ray have returned to Mayo for treatment. Those with sputum positive for cancer cells, but with negative chest x-rays, have been admitted for localization studies, including fiberoptic bronchoscopy. Localization of radiographically occult cancer has been followed by appropriate treatment, usually conservative resection. Several lung cancer risk factors and prognostic factors are also being studied, and special studies relating to the use of hematoporphyrin derivative (Hp-D) as an endobronchial marker for early radiographically unapparent cancer.

Progress: As of February 24, 1976, a total of 10,012 subjects had been interviewed. Initial screening has detected previously unsuspected cancer of the respiratory tract at a rate of approximately 7 per 1,000 interviewed. The "incidence" rate of lung cancer detected following negative initial screening approaches 4 per 1,000 man-years surveillance. Since initiation of the study 130 cases of lung cancer have been diagnosed, and of these, 43 were radiographically occult. Nearly 60% of the 130 cases have been resected, including 20 (74%) of 27 "incidence" cases. The prognosis appears to be good in 42 of the 130 total cases of lung cancer and in 14 (52%) of the 27 "incidence" cases.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Lung Cancer Diagnosis Site Visit Date: 1/13/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $950,000

CONTRACT RESEARCH SUMMARY

Title: Detection of Pancreatic Cancer at Early or Small Stage Prior to Metastasis

Principal Investigator: Vay Liang W. Go., M.D.
Name/Address Mayo Foundation
Performing Organization: Rochester, Minnesota 55901
Contract Number: NO1-CB-43895
Starting Date: 6/15/74 Expiration Date: 6/14/77

Goal: a) To determine comparative values of newer diagnostic tests in the detection of pancreatic cancer; b) To determine whether surgical intervention of a localized pancreatic cancer significantly improves survival rate; and c) To further develop newer procedures for pancreatic cancer diagnosis including the CT body scanner.

Approach: Three cooperating institutions will study, by a common protocol, patients whose symptomatology and initial studies are suspicious of pancreatic cancer (PC) and warrant exploratory laparotomy. The following special tests listed will be performed prior to surgery: Blood studies include CEA, alpha-fetoprotein and immunoglobulins, duodenal aspiration and study of pancreatic enzymes (PFT) and biliary secretion include cytology of duodenal aspirates (Cyt), CEA and other substances of potential diagnostic value for detecting cancer, ultrasonic abdominal study (U), fiberoptic duodenoscopy with retrograde pancreatocholangiography (RPGM), pancreatic scan with ^{75}Selenomethionine (S), selective celiac and/or superior mesenteric angiogram (A), and thermogram (T). At the completion of all the studies, a surgical consultation for possible exploratory laparotomy will be obtained. At operation, portal blood will be obtained for additional studies (including CEA and alpha-fetoprotein). If cancer of the pancreas is found at surgery, the appropriate surgery will be performed and these patients will be evaluated at 3-month intervals for 3 years. If no diagnosis is made at surgery, patient will be put into a follow-up group to be evaluated at 6-month intervals for 3 years.

Progress: To date, 97 patients have been considered for the protocol and 72 patients have been entered into the study. Results of first 59 patients who were surgically explored are as follows: Twenty-eight were found to have PC, 8 other neoplasms, 7 pancreatitis, and 16 benign disease or no organic pathology. A, RPGM, Cyt, and T were judged capable of distinguishing PC from all other disease while U, PFT, and S only distinguish between pancreatic and non-pancreatic diseases. Nonvisualization of pancreas was considered normal for T and U, abnormal for S. Technically unsuccessful tests were only encountered for RPGM (10%) and PFT (9%) and excluded from data analysis. A and RPGM were 67% and 90% accurate in diagnosing PC and were accompanied by 7% and 11% false + rate. U, PFT, and S accurately detected pancreatic disease but S had a high 57% false + rate. The combination of U (a simple non-invasive procedure) to detect pancreatic disease followed by RPGM and A accurately diagnosed PC in 73% of patients and excluded 87% of nonpancreatic disorders. The combination of tests is a potentially useful and practical approach to the diagnosis of PC.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $181,596

CONTRACT RESEARCH SUMMARY

Title: Usefulness of Carcinoembryonic Antigen in the Diagnosis of Bowel Cancer

Principal Investigator: Vay Liang W. Go, M.D.
Name/Address Mayo Foundation
Performing Organization: Rochester, Minnesota 55901

Contract Number: NO1-CB-23854
Starting Date: 6/15/74 Expiration Date: 6/14/77

Goal: a) To evaluate the CEA test in diagnosis of large bowel carcinoma b) continue evaluation of CEA as a biologic marker in patients undergoing chemotherapy, radiotherapy and immunotherapy of advanced gastrointestinal cancer and in patients following resection of gastrointestinal cancer with curative intent. Since renewal of this contract last year, workscope has been expanded to include: c) Measure CEA levels in blood and other specimens stored at NCI-Mayo Clinic Tumor Plasma Bank, other contracts with the Immuno-Diagnostic Committee including those of Emory University and the breast cancer task force cooperative studies; d) To evaluate a newer procedure for screening and diagnosis of large bowel cancer, including hemoccult test and colonic cytology; e) Establish an Inflammatory Bowel Disease Registry of Mayo patients, and f) Collect and provide colonic malignant tissue for different investigators in CEA projects.

Approach and Progress: a) Results of prospective evaluation of serum CEA in health, cancer, and in gastrointestinal secretions were based on 6,700 patients (1,466 were cancer patients of which 757 had GI malignancy, 709 patients had no gastric malignancy, and 5,032 were noncancer patients). b) To date, over 671 patients with GI malignancy are in the study under 20 separate protocols involving different cancer therapies. c) Serum CEA levels have been determined in blood specimens from the NCI-Breast Cancer Cooperative Study, Emory University, NCI-Mayo Clinic Tumor Plasma Bank, and 3,694 patients of the Mayo Lung Cancer Project. d) Protocol was developed for a screening program for detection of early cancer of colon utilizing hemoccult test and colonic cytology. Preliminary results suggest peroxidase activity is not stable in prolonged storage on hemoccult cards. All except specimens containing the highest hemoglobin concentrations (15-38 mg/g stool) became hemoccult-negative within 8 days of storage of hemoccult cards. Hemoccult detection of fecal occult blood was compared with quantitative determinations of GI blood loss after I.V. administration of ^{51}Cr labeled RBC in 80 patients A loss of at least 10 ml/day in ^{51}Cr equivalent was necessary to assure a positive hemoccult reaction. The lavage cytology obtained during enema or pulsating devices were generally not useful. e) 1,213 patients with inflammatory bowel disease have entered into Registry. To date, 410 of these patients returned to Mayo Institution for study and treatment of their disease. f) Total of 19.6 kg of colonic malignant tissue has been collected in 1975 which were distributed to eight different laboratories that have active research projects on CEA.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $195,000

CONTRACT RESEARCH SUMMARY

Title: Detection of Pancreatic Cancer at Early or Small Stage Prior to Metastasis

Principal Investigator: Patrick J. Fitzgerald, M.D.
Name/Address Memorial Hospital for Cancer
Performing Organization: and Allied Diseases
New York, New York 10021

Contract Number: NO1-CB-43975
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: To evaluate diagnositc methods and findings in patients undergoing adominal exploration for supposed pancreatic cancer in order to establish a base that can be used for the detection of small pancreatic cancer before metastasis that may lead to an increase in the 5-year survival from 1% now prevalent to 13% or more.

Approach: Three cooperating institutions will study, in a predetermined manner by a common protocol, patients who are suspected of having cancer of the pancreas and are about to undergo abdominal surgery. Special studies delineated in the protocol will include preoperative and operative study of CEA in peripheral and portal venous blood. Special studies of pancreatic exretions obtained through a fiberoptic scope in the duodenum, pancreatic and biliary ducts will be undertaken. Such secretions will be studied for tumor-associated antigens, abnormal enzyme content, biochemical alterations, as well as cytological changes which will permit the earlier diagnosis of pancreatic cancer.

Progress: In 21 months about 1,050 patients with abdominal complaints were seen by the Gastrenterology Service. 115 of these patients (11%) were suspected of having pancreatic cancer and 54 (47%) were found at operation to have it. Seventeen cases (31%) has "curative" resections 7 of these are living 2-20 months postoperatively. The operative mortality (death within 1 month) was 20%. In 33 other patients, at operation, 26 had non-pancreas cancer and 7 had non-cancerous diseases. Coeliac arteriography was the most helpful diagnostic test. Carcinoma in situ was found in duct epithelium in 20% of cases. Serum CEA values were only helpful in a small percent of cases. Of about 15,000 asymptomatic patients, screened at the Strang Clinic Institute, 15 were proven to have gastrointestinal cancer but no case of cancer of the pancreas was found (about 2 cases were expected from incidence rates).

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Prorgram: Diagnosis Site Visit Date: 1/29/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $165,810

CONTRACT RESEARCH SUMMARY

Title: Lung Cancer - Early Detection, Localization and Therapy

Principal Investigator: Myron R. Melamed, M.D.
Name/Address Memorial Hospital for Cancer
Performing Organization: and Allied Diseases
New York, New York 10021

Contract Number: N01-CN-45007
Starting Date: 9/1/73 Expiration Date: 8/31/76

Goal: To evaluate sputum cytopathology as a supplement to annual chest x-rays in detecting pulmonary neoplasms at an "early stage" and to evaluate the efficacy of techniques for prompt localization of radiologically occult lung cancer (e.g., before progression to x-ray positive); in general the efficacy of such screening to reduce lung cancer mortality.

Approach: Over a 3-year period, 5,000 test subjects and 5,000 control subjects will be entered into this study with active screening for 5 years, all to be followed for at least an additional 5-year period, for sufficient years to be statistically significant to answer pertinent questions. This will be conducted according to a protocol developed in conjunction with the Johns Hopkins University and the Mayo Foundation.

Progress: Subject recruitment began in the latter part of 1974, and as of March 18, 1976, there were a total of 6,612 men enrolled in this program. The study group, having sputum cytology as well as chest x-rays, totaled 3,225; of these, there were seven men with negative x-rays who had lung cancer detected by cytology, 2 who had carcinoma of larynx detected by cytology and 11 with lung cancer detected by x-ray. Of the 3,387 men in the control group having chest x-rays only, there were 12 found with lung cancer. It was of interest that most of the lung cancers detected by cytology with negative chest x-rays were very early; 5 were stage 0, 1 was stage I and 1 stage III.

We are now devoting a major part of our effort and secretarial/clerical time to completing the enrollement, and to maintaining follow-up and compliance with the program by all subjects who have entered it.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Lung Cancer Diagnosis Site Visit Date: 1/30/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 75 Funds: $592,000

CONTRACT RESEARCH SUMMARY

Title: Determination of Optimal Frequency of Screening Strategies

Principal Investigator: Michael J. Brennan, M.D.
Name/Address Michigan Cancer Foundation
Performing Organization: Detroit, Michigan 48201

Contract Number: NO1-CB-53907
Starting Date: 6/1/75 Expiration Date: 5/31/76

Goal: To develop a means whereby the optimal frequency of screening can be determined for early cancer of various anatomic sites in order to assure success in such screening at the least possible cost and hazard to the patient and still be effective in reducing death from cancer.

Approach: A team of clinicians, pathologists, statisticians, operations researchers and epidemiologists will carry out extensive investigation and documentation of the level of present knowledge, both empirical and theoretical on the following five factors: (1) the natural history of development of the disease, (2) the incidence of the disease in the target population, (3) the effectiveness (sensitivity and specificity) of the various available screening tests at each stage of the disease, (4) the impact (on mortality) of intervention (treatment during each stage of the disease) and (5) the cost and hazards of the various screening tests. Disease models will be developed and assessed for sites on which screening data already exists, such as colon, breast and cervical cancer. Lung, bladder and prostatic cancer models will also be undertaken to help define the requisite characteristics of screening techniques for these diseases. Through appropriate statistical and mathematical methods these models will be used to suggest general ranges or minimum limits on the performance of screening tests if they are to be effective in early cancer detection and reducing cancer mortality.

Progress: In the first 6 months of the contract, the following have been accomplished: an organizational structure and functional allocation procedure has been developed that integrates activities of project staff and consultants; the approach to the evaluation of screening strategies has been refined; substantial progress has been made on completing the analysis of current knowledge; Technical Advisory Committees have been established and meetings held for each cancer site; a modeling approach for a colorectal cancer screening model has been identified and procedures to refine existing screening models for breast and cervical cancer established.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 5/3/76
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $114,412

CONTRACT RESEARCH SUMMARY

Title: Detection of Small Amounts (over 7-10 ml per 24 hours) of Human Blood in Human Feces

Principal Investigator: Richard E. Rosenfield, M.D.
Name/Address Mount Sinai School of Medicine
Performing Organization: New York, New York 10029

Contract Number: NO1-CB-43870
Starting Date: 6/1/74 Expiration Date: 5/31/76

Goal: To generate a procedure that will detect small amounts of human blood (over 7-10 ml in 24 hours) in human fecal material by a simple, accurate (98%) examination.

Approach: The contractor will develop a procedure for the detection of human blood in feces by utilizing red blood cell surface antigens specific for human blood. Application of the test will be made with an adequate number of persons both known to be bleeding and thought not to be bleeding; an adequate number of controls must have been on high meat, poultry, and fish diets and be truly negative by the proposed test.

Progress: Successful tests for human blood in feces were developed with artificial mixtures of normal feces containing 10^7, 10^8, and 10^9 erythrocytes per gram. Best results were achieved by diluting 1 g feces in 10 ml distilled water, allowing large particles to settle by low speed centrifugation (500 x g [Sorval GLC-1] for 5 min.), and recovering the supernatant fluid containing light fecal debris and erythrocytic stromal fragments. The latter were then concentrated by high speed centrifugation (30000 x g for 30 minutes [Sorval RC-5]). Pellets of fecal debris and stroma were then suspended in 1 ml 0.9% NaCl, 0.5 ml of which was mixed and incubated at 37° C for 1 hour with an equal volume of anti-Rh29 (RH or "total Rh") diluted in 2% bovine albumin and 0.9% NaCl. Following incubation, the supernatant was recovered after high speed centrifugation and tested for its residual agglutinating activity against ficin treated normal type O red cells. Agglutination, on simple manual test procedure, revealed +++ reactions in the absence of inhibition, ++ after inhibition with 10^7 erythrocytic stroma, + with 10^8, and 0 to ± with 10^9. In parallel guaiac (Hemoccult) tests, these serological results corresponded, respectively, to negative, trace, moderate, and strongly positive. Stool specimens from patients with gastrointestinal bleeding have shown completely concordant results between serological and guaiac tests for colonic bleeding. Blood swallowed by normal volunteers also has been detected readily by both tests, but some bleeding peptic ulcers, possibly in association with high gastric acidity, have given a better result by guaiac than by serology. On the other hand, false positives (high meat) or false negatives (ascorbic acid) by guaiac have not been observed in the serology test.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 12/16/74
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $86,842

CONTRACT RESEARCH SUMMARY

Title: Biochemical Markers or Enzyme Changes That May Presage the Presence of Cancer

Principal Investigator: Joshua Lederberg, Ph.D.
Name/Address Stanford University School of Medicine
Performing Organization: Department of Genetics
Stanford, California 94305

Contract Number: NO1-CB-43902
Starting Date: 6/30/74 Expiration Date: 6/29/76

Goal: To develop further the uses of gas-liquid chromatography and mass spectrometry instrumentation, under computer management, for the study of derangements of human biochemical metabolism due to neoplastic change; this is to be done for the purpose of identifying new metabolites, or altered levels of known metabolites, which might be diagnostic of cancer.

Approach: (a) To screen urine by GC/MS from control subjects and from individuals suffering from the following forms of cancer:
(1) prostatic cancer,
(2) bladder cancer,
(3) Hodgkin's disease, and
(4) various lymphomas.
(b) The quantitation of urinary beta-aminoisobutyric acid levels from cancer patients.
(c) The quantitation of urinary protein amino acid levels in cancer patients.

Progress: A method has been developed for the determination of polyamines in the urine of prostatic cancer patients by Stable Isotope Mass Fragmentography. We have examined 10-15 samples of each of controls, samples from patients with benign prostatic hypertrophy and samples from patients with prostatic cancer. No significant differences were found in the levels of any of the four polyamines (putrescine, cadaverine, spermine, and spermidine) in the above set of samples. The GC/MS profiles of 50 cancer patients has been completed. They are: 8 bladder cancer, 6 non-Hodgkin lymphomas, 5 prostate cancer, 7 leukemias, 6 breast cancer, 6 lung cancer, 6 pancreas cancer, and 6 colon cancer. In addition, a computer program, called CLEANUP, which is designed to detect components and to remove interference from background, column bleed and to resolve overlapping GC peaks, was developed to examine the excretion profiles of cancer patients in more detail. We are assembling computer programs for detailed intercomparison of samples. From our preliminary results beta-aminoisobutyric acid (BAIB) may prove to have diagnostic significance as elevated amounts of BAIB were observed to be excreted in the urine of most of the bladder, prostate, and especially leukemia samples and in three of the six patients with lung cancer and one of the six patients with breast cancer. No other components of diagnostic value have been observed in highly elevated levels. We will soon determine if there are diagnostic markers at lower levels.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 3/22/76
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $95,000

CONTRACT RESEARCH SUMMARY

Title: Biochemical Markers or Enzyme Changes That May Presage Presence of Cancer

Principal Investigators:	Eric A. Barnard, Ph.D.	Tsann Ming Chu, Ph.D.
Name/Address	SUNY at Buffalo	Roswell Park Mem. Inst.
Performing Organization:	Buffalo, N.Y. 14214	Buffalo, N.Y. 14263

Contract Number: NO1-CB-43977
Starting Date: 6/1/74 Expiration Date: 5/31/76

Goal: To develop and establish a highly sensitive test for a specifically prostatic form of serum acid phosphatase as a marker for early prostatic cancer.

Approach: It is intended to prepare in bulk the isoenzymes of human prostatic acid phosphatase, by methods previously proven in this laboratory in small-scale work. Both the water-extracted types, which are believed to be secreted in the prostatic fluid, and the detergent-extracted type (obtained by a Tween method in previous work) will be separately examined. Each will be used as an antigen to obtain antibody and other reagents for a radioimmunoassay method, for its specific detection at very low levels in sera. Each will also be studied for any specific binding or inhibitory property that can characterize it at low levels in the presence of other phosphatases.

Progress: Acid phosphatase isoenzymes from normal, hypertrophic prostate glands and carcinoma of the prostate have been isolated and characterized. Isolation of acid phosphatase from serum of patients with prostatic cancer has also been attempted. Levels of acid phosphatase activity in human prostatic fluids have been studied. Antisera against total prostatic acid phosphatase and purified acid phosphatase isoenzyme have been prepared in rabbits and characterized by immunological techniques. A preparation of purified prostatic acid phosphatase isoenzyme has been iodinated with ^{125}I and a radioimmunoassay is being studied. Over 1,000 serum samples from 140 patients with prostatic cancer at different stages of disease have been collected and stored at -75°C for later assay experiments.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 3/17/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $79,199

CONTRACT RESEARCH SUMMARY

Title: Nuclear Magnetic Resonance in Neoplastic and Non-neoplastic Tissues

Principal Investigator: Raymond Damadian, M.D.
Name/Address Downstate Medical Center, SUNY
Performing Organization: Brooklyn, New York 11203

Contract Number: NO1-CB-43979
Starting Date: 6/15/74 Expiration Date: 6/14/76

Goal: The use of nuclear magnetic resonance spectroscopy for the identification of malignant, neoplastic tissue so that this tissue can be differentiated from normal and non-neoplastic, diseased tissue.

Approach: To conduct blind studies on approximately 2,000 surgical specimens by means of NMR determinations. To determine the extent to which malignant tissue can be detected by NMR in the presence of normal tissue. To explore the feasibility of detecting internal tumors in small laboratory animals.

Progress: NMR studies are being made on four biologically significant atomic nuclei, hydrogen (proton, ^{1}H), potassium (^{39}K), phosphorus (^{31}P) and ^{23}Na (sodium). To date, T_1, T_2 and $T_{1\rho}$ measurements have been made on approximately 600 biological samples. T_1 values of malignant tissues were generally elevated above normal. The difference also appears to hold for the T_2 and $T_{1\rho}$ parameters. NMR studies with ^{39}K, ^{31}P, and ^{23}Na have been limited thus far to tissues from rats and mice. A study of the ^{39}K nucleus is now complete. Tumors are distinguished from normal by the presence of an oscillation in the T_1 plots of tumor tissue. ^{31}P appears to be particularly encouraging. T_1 relaxation times for malignant tumors were 2-3 times the normal values. The degree of overlap in NMR measurements between normal and malignant human surgical tissue is being defined and will affect the value of such measurements for detection and diagnosis.

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 2/5/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $100,000

CONTRACT RESEARCH SUMMARY

Title: Improvement of X-Ray Imaging by Elimination of Scatter, While Retaining the Resolution

Principal Investigator: Gary T. Barnes, Ph.D.
Name/Address University of Alabama
Performing Organization: Birmingham, Alabama 35203

Contract Number: N01-CB-53865
Starting Date: 6/30/75 Expiration Date: 6/29/76

Goal: To remove scatter and thus improve sharpness and contrast which will in turn improve the ability of recognizing tissue differences and thus identifying cancer in its earlier (smaller) stage.

Approach: To construct a scanning multiple slit assembly that will function in a clinically useful range of exposure times and delivered dose and that can obtain the stated primary goals by mechanical means and should eliminate the need for grids and air gaps. Methods will be developed to determine the quality of the films produced as well as relative patient doses required in their production. To be included are realistic measurements of improvements in scatter to primary detected radiation as a function of exposure and subject parameters, in addition to evaluation of resolution, contrast and dose reduction. Feasibility should be established during the first year of this two-year contract.

Progress: Evidence has been obtained indicating that an array of long, narrow beam defining slits scanning a patient coupled with scatter eliminating slots beneath the patient will substantially reduce scatter in diagnostic radiology. This was accomplished by measuring the scatter/primary ratios and the distribution of scatter in the plane of the image detector as a function of slit width and slot depth for a long, narrow beam defining geometry. Using this data, calculations for the scatter/primary ratio incident on the image detector were made for a multiple slit assembly and compared with conventional grids. For the range of tube voltages routinely used in diagnostic radiology, an improvement in contrast is obtained with little or no increase in patient exposure. Such a device has been designed and is presently under construction.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $54,536

CONTRACT RESEARCH SUMMARY

Title: Detection of Pancreatic Cancer at Early or Small Stage Prior to Metastasis

Principal Investigator: A. R. Moossa, M.D.
Name/Address University of Chicago
Performing Organization: Chicago, Illinois 60637

Contract Number: NO1-CB-43976
Starting Date: 6/30/75 Expiration Date: 6/29/76

Progress: To date, 105 patients have been referred to this study. Of these, 5 patients had a positive diagnosis of pancreatic cancer elsewhere and came to our hospital for radiotherapy and/or chemotherapy treatments. Systemic blood was taken from these patients, but no additional investigation was performed. Ninety patients have entered the study. Of these, 62 have been fully investigated and 28 partially. Of the 62 who completed the protocol, 36 have undergone laparotomy. Of these, there were 20 cancers of the pancreas or periampullary region detected - 9 were in the head of the pancreas, 3 in the duodenum, 6 in the body of the pancreas, 1 metastatic cancer of the pancreas, and 1 cancer of the pancreas and the common bile duct. The remaining cases were diagnosed as follows: 1 lymphoma, 1 stricture of the pancreatic duct and common bile duct, 1 cirrhosis, 1 Zollinger-Ellison syndrome, 1 metastatic cancer with undetermined primary site, 2 pancreatic pseudocysts, 7 cases of pancreatitis, and 2 cases of choledocholithiasis. Of the 28 partially investigated patients, 14 underwent laparotomy and the following was found: 6 pancreatic cancers (5 in the head of the pancreas and 1 metastatic adenocarcinoma of the pancreas). The remaining were diagnosed as follows: 2 hiatal hernias, 1 insulinoma, 1 undifferentiated carcinoma of the liver, 1 hemangiopericytoma, 1 fibrous histiocytoma, 1 choledocholithiasis, and finally, 1 negative laparotomy was performed. To date, the most promising diagnostic investigations in our institution appear to be duodenal cytology, endoscopic retrograde cholangiogram and determination of the pancreatic oncofetal antigen level in the blood.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $169,194

CONTRACT RESEARCH SUMMARY

Title: Statistical Center for Cooperative Lung Cancer Groups

Principal Investigator: C. Ralph Buncher, Sc.D.
Name/Address University of Cincinnati Medical Center
Performing Organization: Cincinnati, Ohio 45267

Contract Number: NO1-CB-43868
Starting Date: 6/25/74 Expiration Date: 5/31/79

Goal: To collect and analyze data from the three collaborating lung cancer groups in the lung cancer screening program directed toward the detection of early lung cancer in high risk patients to see if this screening will reduce mortality and morbidity.

Approach: Procedures have been established and agreement has been reached concerning the common data base for this study. The data are being monitored by the Statistical Center, entered into the computer and analyzed. Reports are being made quarterly. The Statistical Center will search the data for significant statistical findings.

Progress: In the Cooperative Early Lung Cancer Group program, the Cincinnati Central Statistical Group (CSG) has established a common data base, made suggestions for uniform data recording by the clinical centers, and reported results to the NCI and participating clinical centers. The CSG has received patient data from each of the clinical centers and formed a master patient file from which tables, reports, and analyses can be derived to answer the questions of the study, i.e., whether efforts directed toward earlier diagnosis have successfully led to improvement in mortality from lung cancer.

Tables showing the results and status at each of the clinics have been produced. They have proven of value to the NCI and other participants as a means of monitoring the progress and results of the study. During this past year a comparison of cytopathology interpretation was conducted. Patient status indicator codes, follow-up records information have been added to the common data base, and computer programs for editing submitted information have been developed.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 12/18/74
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $169,557

CONTRACT RESEARCH SUMMARY

Title: Detection of Small Amounts (over 7-10 ml per 24 hours) of Human Blood in Human Feces

Principal Investigator: Curtis L. Songster, M.D.
Name/Address University of Louisville Foundation, Inc.
Performing Organization: Louisville, Kentucky 40208

Contract Number: NO1-CB-43968
Starting Date: 6/30/74 Expiration Date: 6/19/76

Goal: To generate a procedure that will detect small amounts of human blood (over 7-10 ml in 24 hours) in human fecal material by a simple, accurate (98%) examination.

Approach: The contractor shall develop a procedure for the detection of human blood in feces by utilizing antigens specific for the globin portion (more specifically, alpha chain) of hemoglobin. Application of the test will be made to an adequate number of persons both known to be bleeding and thought not to be bleeding; an adequate number of controls must have been on high meat, poultry, and fish diets and be truly negative by the proposed test.

Progress: High titer goat antisera have been raised against alpha-chain of human hemoglobin. Cow, pig, sheep, chicken and fish hemoglobins lack cross-reaction. Cross-reactions with human hemoglobins are complete for A/A, A/S, A/C, S/S, S/C and monkey; partial with rabbit hemoglobin. Development of a latex agglutination test for human hemoglobin is under way, optimized for experimental variables, including bead preparation, working and final latex concentrations, pH, ionic species and strengths.

Radioimmunoassay for human hemoglobin is under development using two radio-label methods; reactive group coupled with tritiated N-ethyl maleiimide and classical iodination. Radial immunodiffusion techniques using anti-alpha-chain and anti-hemoglobin appear capable of specifically detecting human blood at levels of 1 mg% in extractions of human feces. Extraction methods, potential antigen loss with time, storage changes in feces, and bacterial flora effects are being evaluated in terms of any influence upon specific immunoassays.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 4/13/76
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 76 Funds: $60,678

CONTRACT RESEARCH SUMMARY

Title: Impact of Multi-Site Screening on Total Cancer Mortality

Principal Investigator: Victor A. Gilbertsen, M.D.
Name/Address University of Minnesota
Performing Organization: Minneapolis, Minnesota 55455

Contract Number: NO1-CB-53906
Starting Date: 6/30/75 Expiration Date: 6/29/76

Goal: The goal of this project is to design a multi-site model put to clinical use in order to effectively reduce cancer mortality.

Approach: A team will be established with expertise in the fields of clinical oncology, radiology, epidemiology and statistics. The experimental design will be developed on the basis of available knowledge of: 1) high-risk factors, 2) quality of screening procedures, 3) variability in the effectiveness of present day treatment, 4) cost, 5) compliance with follow-up examinations and follow through procedures, 6) side effects of treatments, 7) incidence rates, 8) prevalence rates and 9) case fatality rates. The rationale for the experimental design will be expressed in a format which makes explicit the use of available information and accepted assumptions.

Progress: Literature review has included available basic factual information related to concepts of the role of screening for neoplastic disease in health care delivery as well as search of the periodical literature. Attention is being directed to a number of areas including incidence and prevalence rates of the several cancers, risk factors, identification of available "screening procedures," sensitivity and specificity of such procedures, acceptability of screening procedures to the population, lead time evaluation, estimates of duration of "preclinical" disease, information concerning survival related to earlier diagnosis, and availability and effectiveness of present-day treatments. Particular attention has been directed to data concerning cancers of the breast, colon-rectum, prostate gland, lung, and uterus. The concept of multiphasic screening and experience reported therewith has also received attention.

Data available to the investigators from the 27-year experience of the Cancer Detection Center at the University, although largely unpublished, are also in the process of evaluation. Pertinent aspects of these data are being carefully analyzed and further computer assisted analysis is under way.

Periodic meetings have been conducted with those knowledgeable in the several areas where the likelihood of the feasibility of screening for detection of earlier cancers would appear to exist. Such meetings have included discussion of pertinent data as related to the several anatomic sites of cancer as well as to the concept of Multi-Site Cancer Screening and are continuing at regular intervals to permit further progress toward formulation of Multi-Site Screening models.

Significance for Cancer Research (NCP Objective 5 Approach 3)
Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $69,212

CONTRACT RESEARCH SUMMARY

Title: Screening Technique for Blood in Stool To Detect Early Cancer of Bowel

Principal Investigator: Victor A. Gilbertsen, M.D.
Name/Address University of Minnesota
Performing Organization: Minneapolis, Minnesota 55455

Contract Number: NO1-CB-53862
Starting Date: 6/30/75 Expiration Date: 6/29/76

Goal: The goal of this project is to increase the five-year survival of the patients with large bowel cancer.

Approach: After high risk characteristics are established with the aid of biostatisticians and epidemiologists, patients will be entered into the study in sufficient numbers to give valid comparisons. A control cohort will also be established. Clinical facilities will also be established for diagnosis, treatment, and follow-up of large bowel cancer patients. Suitable forms will be designed for conversion to computer storage and subsequent retrieval for evaluation of predisposing factors. The development of a more sophisticated test for human blood will also lead to the design of a common protocol with the collaborating contractor.

Progress: The occult blood screening project to screen 30,000 persons between 50 and 80 years of age for occult blood in the stool, employing the guaiac test (Hemoccult). Persons producing a positive test for stool blood complete while observing the meat-free restricted diet are subject to a complete diagnostic sequence at the University of Minnesota Health Sciences Center to determine the cause of bleeding.

Participants randomized to all three groups will begin receiving an extensive dietary and medical history questionnaire during the first week of April 1976. Follow-up of control group subjects will be initiated concurrently. The questionnaire is designed to elicit extensive, medical, demographic, socioeconomic, and early environmental and family history on each participant. Additional medical history data will be requested for the participant himself.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $308,437

CONTRACT RESEARCH SUMMARY

Title: Methods of Bowel Preparation Preparatory to Barium Enema or Colonoscopy

Principal Investigator: Harry W. Fischer, M.D.
Name/Address University of Rochester
Performing Organization: School of Medicine and Dentistry
Rochester, New York 14642

Contract Number: NO1-CB-43896
Starting Date: 6/1/74 Expiration Date: May 31, 1976

Goal: To perfect methods for insuring a clean colon for the benefit of the radiologist and/or the endoscopist. The technique should not require long periods of time (within a 24-hour period) and without excessive dietary restrictions and without excessive patient discomfort or inconvenience.

Approach: To put to trial combinations of some older methods, or combinations of certain features of older methods, along with the testing of a previously little used method of assisting conversion of solid stool to semi-solid or liquid phase. It is proposed that fecal material be converted to a more liquid form (Embring) and the patient then be assisted to evacuate. The entrance of additional material into the colon from above would be minimized by a certain degree of dietary restriction prior to the definitive portions of the technique aimed at stool conversion and evacuation.

Progress: Evaluation of toxicity of ammonium alum and potassium alum for possible use as a cleansing agent for barium enema has progressed through gross and histological study of rat tissue, and an enzyme study in rats, rabbits, and dogs. Electrolyte studies in dogs have also been performed. No significant abnormality in serum enzyme and electrolyte values has been found after employment of 1% alum as a one-hour retention enema. Histological studies of rabbits and dogs: liver and kidney are being completed. A study of serum enzymes and electrolytes in rabbits with experimentally induced ulcerated colons is in progress. A new method of cleansing the human bowel for barium enema has been put to test in a small number of human volunteers. This method consists of flushing the intestinal tract with hypotonic balanced salt solution until clear liquid bowel movements indicate the colon is completely cleansed. This has been found successful in most individuals and could be quite useful as an alternative method for bowel preparation.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 3/18/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnostic Steering Committee
FY 75 Funds: $74,267

CONTRACT RESEARCH SUMMARY

Title: Design of an Experiment to Assess the Impact of Multi-site Screening on Total Cancer Mortality

Principal Investigator: Dean F. Davies, M.D.
Name/Address University of Tennessee
Performing Organization: Memphis, Tennessee 38163

Contract Number: NO1-CB-53864
Starting Date: 6/23/75 Expiration Date: 6/22/76

Goal: The goal of this project is to achieve a design that can be put to clinical use in order to effectively reduce cancer mortality.

Approach: A team has been established with expertise in the fields of clinical oncology, radiology, epidemiology, and statistics. The experimental design will be developed on the basis of available knowledge of: 1) high risk factors, 2) quality of screening procedures, 3) variability in the effectiveness of present day treatment, 4) cost, 5) compliance with follow-up examinations and follow through procedures, 6) side effects of treatments, 7) incidence rates, 8) prevalence rates and 9) case fatality rates. The rationale for the experimental design will be expressed in a format which makes explicit the use of available information and accepted assumptions.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $104,110

CONTRACT RESEARCH SUMMARY

Title: Improvement of X-Ray Imaging by Elimination of Scatter While Retaining the Resolution

Principal Investigator: Melvin P. Siedband, M.S.
Name/Address University of Wisconsin
Performing Organization: Madison, Wisconsin 53706

Contract Number: NO1-CB-53914
Starting Date: 6/30/75 Expiration Date: 12/29/77

Goal: To remove scatter and thus improve sharpness and contrast which will in turn improve the ability of recognizing tissue differences and thus identifying cancer in its earlier (smaller) stage.
Approach: To construct a system that will function in a clinically useful range of exposure times and delivered dose and that can obtain the stated primary goals by mechanical and/or electronic means and should eliminate the need for grids and air gaps. Methods will be developed to determine the quality of the films produced as well as relative patient doses required in their production. To be included are realistic measurements of improvements in scatter to primary detected radiation as a function of exposure and subject parameters, in addition to evaluation of resolution, contrast and dose reduction. Feasibility should be established during the first year of this contract.
Progress: A moving paired slit system has been tested for its tracking ability by looking at the difference signal from the position-sensitive potentiometers on the two slits. For a typical exposure time of 1 second (a speed of about 30 cm/sec at the bottom slit), the maximum fluctuation in tracking was about 0.5 mm in the position of the lower slit within overall limits of 1 mm. The same kind of accuracy is observed at other speeds.

The system was initially tested on a single-phase x-ray machine which produces an x-ray beam which fluctuates at 120 Hz. This was very useful to test the timing accuracy of the system, as the modulation of the intensity was easily visible in test radiographs.

A simple tool to test the ability of the system to reduce scattered radiation was constructed. It consists of a series of different-width lead strips (0.2 cm to 3 cm wide) mounted on a lucite sheet to be placed on top of a uniform lucite phantom.

Two approaches have been made to study the nature of the scattered radiation produced by a uniform phantom in an x-ray field. The first comes from an analysis of Monte Carlo calculations done by Reiss & Steinle†, the second from direct measurements using a stationary slit. Direct measurement of the scattered radiation distribution is also being studied.
Significance for Cancer Research (NCP Objective 5 Approach 4)
Project Officer: Louis G. Greenberg
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $121,946

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Harold J. Isard, M.D. — Marc Lapayowker, M.D.
Name/Address — Albert Einstein Med. Ctr. — Temple University Hosp.
Performing Organization: Philadelphia, PA 19141 — Philadelphia, PA 19140

Contract Number: N01-CN-45058
Starting Date: 1/2/74 Expiration Date: 1/1/77

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening		Early Re-Exams (1-11 Months)		Regular Re-Exams (12 Months)	
	Einstein	Temple	Einstein	Temple	Einstein	Temple
Total Patients	5,018	4,913	592	758	2,426	1,480
Biopsies Recommended	134	176	36	63	44	33
Biopsies Performed	85	92	18	16	27	6
Early Re-Exam Recommended	806	1,245	112	184	140	168
Number of Verified Cancers	17	15	3	2	6	2

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 3/5/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $269,262

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Ned D. Rodes, M.D.
Name/Address Cancer Research Center
Performing Organization: Columbia, Missouri 65201

Contract Number: NO1-CN-45095
Starting Date: 6/1/74 Expiration Date: 6/1/77

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	8,239	974	2,477
Biopsies Recommended	197	50	13
Biopsies Performed	162	25	5
Early Re-Exam Recommended	1,521	99	269
Number of Verified Cancers	33	6	1

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 1/14/76
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $335,000 (Estimated)

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Benjamin Rush, M.D.
Name/Address College of Med. and Dentistry of New Jersey
Performing Organization: Newark, New Jersey 07103

Contract Number: NO1-CN-35006
Starting Date: 6/28/73 Expiration Date: 11/30/76

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	10,152	1,368	3,750
Biopsies Recommended	141	38	26
Biopsies Performed	54	9	-
Early Re-Exam Recommended	2,310	198	154
Number of Verified Cancers	21	2	-

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 1/24/76
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $265,161

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Robert McLelland, M.D.
Name/Address Duke University
Performing Organization: Durham, North Carolina 27710

Contract Number: NO1-CN-45064
Starting Date: 2/15/74 Expiration Date: 11/1/76

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	10,073	1,667	4,182
Biopsies Recommended	267	44	44
Biopsies Performed	194	27	25
Early Re-Exam Recommended	2,571	374	588
Number of Verified Cancers	22	4	2

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 10/7/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $227,715

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

	Robert L. Brown, M.D.	
Principal Investigator:	Robert L. Egan, M.D.	A. Hamblin Letton, M.D.
Name/Address	Emory University	Georgia Baptist Hosp.
Performing Organization:	Atlanta, Georgia 30322	Atlanta, Georgia 30312

Contract Number: NO1-CN-55277 (Successor to NO1-CN-35016)
Starting Date: 5/9/73 Expiration Date: 6/9/77

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening		Early Re-Exams (1-11 Months)		Regular Re-Exams (12 Months)	
	Emory	Georgia B	Emory	Georgia B	Emory	Georgia B
Total Patients	2,075	8,088	237	693	1,391	5,366
Biopsies Recommended	52	333	19	34	23	100
Biopsies Performed	60	173	17	34	14	19
Early Re-Exam Recommended	260	1,112	91	39	106	201
Number of Verified Cancers	7	30	3	8	1	3

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: G.B. 12/3/74
E.U. 12/4/74

Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $265,000 (Estimated)

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Bruce Shnider, M.D.
John Potter, M.D.
Name/Address Georgetown University
Performing Organization: Washington, D.C. 20007

Contract Number: NO1-CN-45062
Starting Date: 2/7/74 Expiration Date: 2/6/77

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	8,396	78	193
Biopsies Recommended	383	14	9
Biopsies Performed	45	5	-
Early Re-Exam Recommended	1,686	33	23
Number of Verified Cancers	20	-	-

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 10/2/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $265,109

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Morton J. Goodman, M.D.
Name/Address Good Samaritan Hosp. and Med. Center
Performing Organization: Portland, Oregon 97201

Contract Number: N01-CN-45088
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	8,072	1,113	2,613
Biopsies Recommended	346	91	32
Biopsies Performed	172	27	7
Early Re-Exam Recommended	1,343	383	65
Number of Verified Cancers	21	5	1

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 1/9/76
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $250,000 (Estimated)

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Philip Strax, M.D.
Name/Address Guttman Breast Institute
Performing Organization: New York, New York 10013

Contract Number: NO1-CN-55306 (Successor to NO1-CN-35004)
Starting Date: 4/18/73 Expiration Date: 6/30/77

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	9,682	130	7,704
Biopsies Recommended	247	4	127
Biopsies Performed	119	9	78
Early Re-Exam Recommended	1,752	68	1,576
Number of Verified Cancers	25	3	19

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 10/4/74
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $400,000 (Estimated)

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Donald C. Young, M.D.
Name/Address Iowa Lutheran Hospital
Performing Organization: Des Moines, Iowa 50316

Contract Number: NO1-CN-45067
Starting Date: 2/27/74 Expiration Date: 6/26/77

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	6,577	216	1,339
Biopsies Recommended	271	23	28
Biopsies Performed	54	5	-
Early Re-Exam Recommended	501	11	-
Number of Verified Cancers	8	-	-

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 10/8/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $240,000 (Estimated)

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Loren J. Humphrey, M.D., Ph.D.
Name/Address Kansas Univ. Med. Center
Performing Organization: Kansas City, Kansas 66103

Contract Number: NO1-CN-55303 (Successor to NO1-CN-35026)
Starting Date: 6/19/73 Expiration Date: 6/18/77

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	10,003	2,856	4,427
Biopsies Recommended	304	47	30
Biopsies Performed	192	20	12
Early Re-Exam Recommended	3,600	2,633	634
Number of Verified Cancers	39	1	2

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 1/23/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $300,000 (Estimated)

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: James E. Youker, M.D.
Name/Address The Medical College of Wisconsin
Performing Organization: Milwaukee, Wisconsin 53226

Contract Number: NO1-CN-55308 (Successor to NO1-CN-35018)
Starting Date: 6/18/73 Expiration Date: 6/17/77

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	9,557	1,627	5,035
Biopsies Recommended	300	93	109
Biopsies Performed	180	50	40
Early Re-Exam Recommended	1,704	1,489	293
Number of Verified Cancers	48	9	8

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 10/2/74
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $310,000 (Estimated)

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Elisabeth Ward, M.D.
Name/Address Mountain States Tumor Institute
Performing Organization: Boise, Idaho 83702

Contract Number: NO1-CN-55305 (Successor to NO1-CN-35029)
Starting Date: 6/25/73 Expiration Date: 6/30/77

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	10,256	1,977	6,473
Biopsies Recommended	191	122	49
Biopsies Performed	209	91	31
Early Re-Exam Recommended	1,712	455	192
Number of Verified Cancers	42	9	5

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 1/15/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $300,000 (Estimated)

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Fred I. Gilbert, Jr., M.D.
Name/Address Pacific Health Research Institute
Performing Organization: Honolulu, Hawaii 96813

Contract Number: NO1-CN-45046
Starting Date: 12/19/73 Expiration Date: 12/18/76

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	10,076	1,890	3,919
Biopsies Recommended	409	240	228
Biopsies Performed	392	103	97
Early Re-Exam Recommended	3,117	1,332	496
Number of Verified Cancers	33	10	8

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 6/12/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $252,544

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Herbert P. Constantine, M.D.
Name/Address Rhode Island Hospital
Performing Organization: Providence, Rhode Island 02902

Contract Number: NO1-CN-45096
Starting Date: 5/28/74 Expiration Date: 7/23/76

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	6,887	347	81
Biopsies Recommended	87	8	-
Biopsies Performed	51	3	-
Early Re-Exam Recommended	843	62	1
Number of Verified Cancers	14	1	-

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 4/7/76
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $244,365

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Duncan L. Moore, M.D.
Name/Address St. Joseph's Hospital
Performing Organization: Houston, Texas 77002

Contract Number: NO1-CN-55100
Starting Date: 10/1/74 Expiration Date: 10/31/76

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	6,837	54	1
Biopsies Recommended	263	5	-
Biopsies Performed	141	2	-
Early Re-Exam Recommended	383	7	-
Number of Verified Cancers	22	-	-

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 4/8/76
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $273,800

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Marvin V. McClow, M.D.
Name/Address St. Vincent's Medical Center
Performing Organization: Jacksonville, Florida 32202

Contract Number: NO1-CN-55210
Starting Date: 6/20/73 Expiration Date: 1/27/77

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	10,083	1,205	4,974
Biopsies Recommended	317	43	33
Biopsies Performed	223	38	29
Early Re-Exam Recommended	1,335	358	268
Number of Verified Cancers	47	6	5

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 3/7/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $252,500

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Robert Schweitzer, M.D.
Name/Address Samuel Merritt Hospital
Performing Organization: Oakland, California 94609

Contract Number: NO1-CN-45068
Starting Date: 2/25/74 Expiration Date: 3/23/77

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	9,323	1,284	2,367
Biopsies Recommended	1,116	288	181
Biopsies Performed	171	20	12
Early Re-Exam Recommended	2,123	538	282
Number of Verified Cancers	35	1	3

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 10/3/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $304,147

CONTRACT RESEARCH SUMMARY

Title: Data Management Center for Breast Cancer Demonstration Projects

Principal Investigator: Perry Sheinock, Ph.D.
Name/Address University City Science Center
Performing Organization: Philadelphia, Pennsylvania 19104

Contract Number: NO1-CN-45161 (Successor to NO1-CN-35035)
Starting Date: 6/28/74 Expiration Date: 6/27/77

Goal: To operate a Data Management Center for the Breast Cancer Demonstration Projects.

Approach: 1) Design, implement, and maintain a computer system for the collection, editing, storage, and retrieval of all data collected in the Demonstration Projects. 2) Assume the responsibility for the receipt and control of all documents and other materials transmitted to the Data Management Center by the Demonstration Projects, the Data Conversion Centers, the Computer Center, NCI, ACS, and any other institution forwarding patient data to the Data Management Center. 3) Assure the completeness and accuracy of all records with manual and computer edit procedures. 4) Maintain records, provide listings, provide reports, provide manuals, and carry out other miscellaneous operations in order to fulfill its function as a Data Management Center.

Progress: In the course of this contract, the over-all data handling system was designed and implemented. A forms inventory procedure was started, microfilming techniques developed, and all manual operations improved to make them routine. Data are edited with the computer, the master file is being formed, and mailing labels for patient recall notification are furnished to the screening centers on a monthly basis. Data are received on a regular basis from the centers and data handling within the DMC is kept current. A Computer File Change Document, to provide a means for the screening centers to correct file errors, has been developed and is being implemented. Details of data management are under constant review with the individual centers. A revised Procedures Manual for the Data Management Center was developed in November 1975.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and Mr. Theodore Weiss
Program: Cancer Control Site Visit Date: 1/10/74
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 76 Funds: $800,000 (Estimated)

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Arthur J. Present, M.D.
Name/Address University of Arizona
Performing Organization: Tucson, Arizona 85724

Contract Number: N01-CN-55097
Starting Date: 10/9/74 Expiration Data: 1/7/77

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	4,457	138	-
Biopsies Recommended	123	5	-
Biopsies Performed	41	-	-
Early Re-Exam Recommended	1,024	22	-
Number of Verified Cancers	3	-	-

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $284,933

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Myron Moskowitz, M.D.
Name/Address University of Cincinnati
Performing Organization: College of Medicine
Cincinnati, Ohio 45229
Contract Number: NO1-CN-55310 (Successor to NO1-CN-35024)
Starting Date: 6/28/73 Expiration Date: 6/27/77

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	10,970	3,455	6,774
Biopsies Recommended	1,213	602	451
Biopsies Performed	630	196	141
Early Re-Exam Recommended	3,795	1,462	920
Number of Verified Cancers	82	10	15

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 12/6/74
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $305,000 (Estimated)

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Condict Moore, M.D.
Name/Address University of Louisville
Performing Organization: Louisville, Kentucky 40201

Contract Number: NO1-CN-55307 (Successor to NO1-CN-35012)
Starting Date: 4/17/73 Expiration Date: 6/30/77

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	10,237	1,480	5,345
Biopsies Recommended	841	108	169
Biopsies Performed	261	45	33
Early Re-Exam Recommended	1,901	246	369
Number of Verified Cancers	40	5	6

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 12/5/74
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $300,000 (Estimated)

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Barbara Threatt, M.D.
Name/Address University of Michigan
Performing Organization: Ann Arbor, Michigan 48104

Contract Number: NO1-CN-45049
Starting Date: 3/18/74 Expiration Date: 6/17/77

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	6,673	904	1,306
Biopsies Recommended	227	24	21
Biopsies Performed	219	15	-
Early Re-Exam Recommended	1,405	845	64
Number of Verified Cancers	41	5	-

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 10/9/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $290,000 (Estimated)

CONTRACT RESEARCH SUMMARY

Title: Thermography Technologist Training Program

Principal Investigator: JoAnn D. Haberman, M.D.
Name/Address University of Oklahoma
Performing Organization: Oklahoma City, Oklahoma 73190

Contract Number: NO1-CN-55163
Starting Date: 9/9/74 Expiration Date: 11/8/76

Goal: To train thermography technicians to improve the quality of thermograms obtained in breast examination in order to enhance the ability to demonstrate early cancer.

Approach: A training program for thermographic technicians involved in the A.C.S.-N.C.I. Breast Cancer Demonstration Projects has been established. The course is for one week, and no more than two technicians are scheduled in any one week. This arrangement provides teaching on a one-to-one basis. Study carrels with audio-tape slides are available as part of the currriculum. The trainee spends approximately 20 hours with the University of Oklahoma technicians in the screening center to observe techniques and carry them out. In addition there are 15 hours of tape/slide lectures and 5 hours of lectures on techniques. Conferences with the Breast-Screening physicians, bioengineers and technologists also are arranged to provide an opportunity for questions.

A method of evaluating the effectiveness of the course is being used. A thermographic technique book will be available identifying the various technical problems to be encountered and their resolution.

Progress: As of April 1976, 21 students from 15 projects have attended the course. The average score on the pre-test before entering the course was 69 out of a possible 100. Post-test average is 94 out of a possible 110.

Significance for Cancer Research: (NCP Objective 5 Approach 4)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Ad hoc Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $46,000

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: JoAnn D. Haberman, M.D.
Name/Address University of Oklahoma
Performing Organization: Health Science Center
Oklahoma City, Oklahoma
Contract Number: NO1-CN-55309 (Successor to NO1-CN-35020)
Starting Date: 6/18/73 Expiration Date: 6/17/77

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	10,212	2,177	5,336
Biopsies Recommended	151	105	169
Biopsies Performed	125	41	27
Early Re-Exam Recommended	2,247	570	595
Number of Verified Cancers	42	6	3

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 1/17/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $315,000 (Estimated)

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Bernard Fisher, M.D.
Name/Address University of Pittsburgh
Performing Organization: Pittsburgh, Pennsylvania 15261

Contract Number: NO1-CN-45065
Starting Date: 2/15/74 Expiration Date: 6/14/77

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	5,965	1,126	248
Biopsies Recommended	115	50	1
Biopsies Performed	12	7	-
Early Re-Exam Recommended	2,213	198	-
Number of Verified Cancers	3	1	-

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 10/10/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $270,000 (Estimated)

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Lewis W. Guiss, M.D.
Name/Address University of Southern California
Performing Organization: Los Angeles, California 90033

Contract Number: NO1-CN-45098
Starting Date: 6/25/74 Expiration Date: 6/24/77

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	7,565	972	508
Biopsies Recommended	320	451	24
Biopsies Performed	23	54	-
Early Re-Exam Recommended	1,659	474	24
Number of Verified Cancers	4	9	-

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 1/12/76
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $330,000 (Estimated)

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Henry Burko, M.D.
Name/Address Vanderbilt University School of Medicine
Performing Organization: Nashville, Tennessee 37232

Contract Number: NO1-CN-55099
Starting Date: 7/24/74 Expiration Date: 7/23/77

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	8,909	166	84
Biopsies Recommended	195	22	-
Biopsies Performed	27	2	-
Early Re-Exam Recommended	486	62	3
Number of Verified Cancers	9	-	-

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 4/9/76
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $248,610

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Thomas Carlile, M.D.
Name/Address Virginia Mason Medical Center
Performing Organization: Seattle, Washington 98101

Contract Number: NO1-CN-55304 (Successor to NO1-CN-35028)
Starting Date: 6/15/73 Expiration Date: 6/15/77

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	10,233	1,813	6,837
Biopsies Recommended	163	73	53
Biopsies Performed	173	59	36
Early Re-Exam Recommended	1,895	1,185	267
Number of Verified Cancers	61	11	12

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 1/14/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $310,000

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Leslie W. Whitney, M.D.
Name/Address Wilmington Medical Center
Performing Organization: Wilmington, Delaware 19805

Contract Number: NO1-CN-45047
Starting Date: 1/1/74 Expiration Date: 12/31/77

Goal: To have the American Cancer Society through its volunteers recruit 5,000 women in the first year and an additional 5,000 in the second year to come to the project for thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for a total of five screenings and to be followed annually for five years thereafter.

Approach: Each of 27 projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography and physical examination so that non-palpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: As of January 1976, results reported from the project to the data management center were as follows:

	Initial Screening	Early Re-Exams (1-11 Months)	Regular Re-Exams (12 Months)
Total Patients	9,957	1,959	4,337
Biopsies Recommended	212	54	33
Biopsies Performed	226	52	36
Early Re-Exam Recommended	2,332	616	252
Number of Verified Cancers	48	5	9

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D. and James E. Hamner, D.D.S., Ph.D.
Program: Cancer Control Site Visit Date: 10/1/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 76 Funds: $265,415

CONTRACT RESEARCH SUMMARY

Title: Development of a Computerized Transaxial X-Ray Reconstruction System

Principal Investigator: Jay A. Stein, Ph.D.
Name/Address
Performing Organization: American Science and Engineering, Inc.
955 Massachusetts Avenue
Cambridge, Massachusetts 02139

Contract Number: NO1-CB-53858
Starting Date: 6/30/75 Expiration Date: 6/29/77

Goal: To establish a new radiologic process that will contribute toward earlier and more accurate detection, identification, and localization of cancer pathology in the chest, abdomen, and other anatomical regions, with potential low risk to the patient.

Approach: This study involves two cooperative contracts (NO1-CB-53858 and NO1-CB-53974). In the initial year, equipment and displays will be developed and fabricated for computerized transaxial x-ray reconstruction for parts of the torso analogous to present equipment used for such studies of the head. At the same time, suitable algorithms for radiologic cross-section images of high spatial densitometric and temporal resolution will also be developed. In the ensuing two years, clinical trials of equipment will be pursued on suitable human subjects and results compared with conventional diagnostic radiological and clinical examinations taken on the same subjects. Instrumentation and algorithms modifications suggested by engineering evaluations and clinical experience will be incorporated as warranted and feasible. Independent appraisal of the radiographic results and diagnostic success will be made by a panel of experts selected by the National Cancer Institute.

Progress: The body scanner design configuration has been completed and fabrication of the unit is well under way. Major components are scheduled for assembly and testing during spring of 1976. Development of suitable algorithms has progressed well and the best two reconstruction techniques are being refined for initial clinical testing. Installation of the body scanner unit at Presbyterian Hospital, for evaluation under Contract NO1-CB-53974, is scheduled for summer of 1976.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Dates: 11/20/74; 5/5/76
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $1,000,000

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator: Sadek K. Hilal, M.D., Ph.D.
Name/Address Columbia University
Performing Organization: College of Physicians and Surgeons
New York, New York 10032

Contract Number: NO1-CB-43910
Starting Date: 6/30/74 Expiration Date: 6/29/77

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide scan, angiography and pneumoencephalography.

Approach: Five (5) cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the x-ray absorption characteristics of normal brain. The number of patients available in this study will provide statistically significant information.

Progress: As of February 1976, 169 patients have been studied including 163 Group I (60 Group IA, 54 Group IB, and 49 Group IC) and 6 Group II.

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 2/18/75
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $106,322

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator: D. Gordon Potts, M.D.
Name/Address Cornell University Medical College
Performing Organization: New York, New York 10021

Contract Number: NO1-CB-43980
Starting Date: 6/30/74 Expiration Date: 6/29/77

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide brain scan, angiography and pneumoencephalography.

Approach: Five (5) cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the x-ray absorption characteristics of normal brain. The number of patients available in this study will provide statistically significant information.

Progress: As of February 1976, 381 patients in Group I, 27 patients in Group II, and 22 normal subjects (total 430) have been studied. These patients are referred for study by the Department of Neurology or Neurosurgery at New York Hospital and by Memorial Hospital. The isotope scans, EMI scans, angiograms, pneumograms and skull films are being performed using projections that can be readily compared. The magnification and degree of distortion of every view may be determined. Patients who come to autopsy are having giant histological sections prepared to show the entire brain in the planes of the EMI sections; 10 cases have been studied in this manner. This will permit an accurate comparison of the EMI views with the other studies.

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 2/19/75
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $111,000

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator: David O. Davis, M.D.
Name/Address George Washington University
Performing Organization: Washington, D.C. 20006

Contract Number: NO1-CB-43981
Starting Date: 6/30/74 Expiration Date: 6/29/77

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide brain scan, angiography, and pneumoencephalography.

Approach: Five (5) cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the x-ray absorption characteristics of normal brain. The number of patients available in this study will provide statistically significant information.

Progress: As of February 1976, 226 patients have been studied; this number includes 174 in Group I (110 Group IA, 44 Group IB, and 20 Group IC); 42 in Group II; and 10 normals (Group III).

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 2/14/75
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $90,293

CONTRACT RESEARCH SUMMARY

Title: Algorithms for Computerized Transaxial Nuclide Reconstruction

Principal Investigator: John A. Correia, Ph.D.
Name/Address Massachusetts General Hospital
Performing Organization: Boston, Massachusetts 02114

Contract Number: NO1-CB-53913
Starting Date: 6/30/75 Expiration Date: 6/29/77

Goal: To improve detection and diagnosis of early cancer lesions using radioactive compounds.

Approach: Computerized transaxial nuclide reconstruction based on transverse section emission data from human subjects appears particularly promising for use with structure-specific labeling with a wide array of radioactive compounds. Emission imaging may permit not only static reconstruction but also quantitative estimates of time-course movements of labeled compounds through structures located deep within the body. Transaxial reconstruction will permit precise estimates of location and intensity of radioactivity in small body volumes that would be obscured by surrounding radioactivity and internal radioabsorption in conventional viewing techniques. In the present project, algorithms will be developed for computerized reconstruction of clinical data produced by novel transaxial nuclide projection machines. Novel radionuclide imaging systems and appropriate algorithms will be emphasized. These algorithms, written in a high level programming language such as FORTRAN, together with appropriate phantom, animal and human projection data and reconstructions, will be recorded on nine track industry compatible magnetic tape and delivered to the National Cancer Institute for independent review and evaluation and dissemination.

Progress: Analytical methods and data collection protocols developed during the initial six months of the study now are in use with the scintillation camera and the positron cameras developed by the research group. Phantom studies and simulations are being used to test influence of many factors on image reconstruction. Rotating tables have been constructed for both scintillation and positron studies with phantoms and with human subjects.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 3/29/76
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $69,135

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator: Paul F. J. New, M.D.
Name/Address Massachusetts General Hospital
Performing Organization: Boston, Massachusetts 02114

Contract Number: NO1-CB-43983
Starting Date: 6/30/74 Expiration Date: 6/29/77

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide brain scan, angiography and pneumoencephalography.

Approach: Five (5) cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the x-ray absorption characteristics of normal brain. The number of patients available in this study will provide statistically significant information.

Progress: As of February 1976, 116 subjects have been studied; this includes 91 in Group I (47 Group IA; 17 Group IB; and 27 Group IC), 16 in Group II, and 9 in Group III.

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 2/13/75
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $120,918

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator: Hillier L. Baker, M.D.
Name/Address Mayo Foundation
Performing Organization: Rochester, Minnesota 55901

Contract Number: NO1-CB-43982
Starting Date: 6/15/74 Expiration Date: 6/14/77

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide brain scan, angiography, and pneumoencephalography.

Approach: Five (5) cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the x-ray absorption characteristics of normal brain. The number of patients available in this study will provide statistically significant information.

Progress: As of December 31, 1975, 313 patients have been admitted into the study including 233 Group I (118 Group IA, 65 Group IB, 49 Group IC, and 1 not completed), 30 Group II, and 50 Group III. Among the 118 Group IA patients, 75% had brain neoplasms, 11% had non-structural intracranial disease, and 13% had non-tumorous structural intracranial disease. Corresponding distributions among the 65 Group IB patients were 45%, 23%, and 28% and among the 49 Group IC patients, 12%, 68%, and 18%. Among the 30 patients in Group II, 87% have proven no evidence of intracranial metastases; the remaining 13% have an indeterminate diagnosis but show evidence of structural disease. None of the 50 patients in Group III were found to have brain abnormalities.

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 1/16/75
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $133,285

CONTRACT RESEARCH SUMMARY

Title: Data Base for Testing Algorithms for Computerized Transaxial Reconstruction

Principal Investigator: Richard A. Robb, Ph.D.
Name/Address Mayo Foundation
Performing Organization: Rochester, Minnesota 55901

Contract Number: NO1-CB-53857
Starting Date: 6/30/75 Expiration Date: 6/29/76

Goal: To obtain information which can be used by radiologists to expedite establishment of new computerized evaluation procedures for improved x-ray detection of early cancer lesions.

Approach: A transaxial x-ray projection data base suitable for testing computerized reconstruction algorithms will be assembled. The data base will include five distinct classes of transaxial planar projection information: simulated data and data from phantoms, excised organs and body specimens, live animals and human subjects. Real projection data will be obtained using x-ray sources and detectors of established physical characteristics and various geometries under controlled experimental conditions. Simulated projection data will be mathematically and stochastically defined in accordance with explicit anatomical, biological, and radiological models of normal and pathological physiologic states and of the data acquisition process. Projection data will be digitized and recorded on industry compatible magnetic tape and delivered to the National Cancer Institute, along with appropriate documentation. The National Cancer Institute will furnish the data to other contractors for evaluation, improvement, and comparison of reconstruction algorithms.

Progress: All written test, simulation, and tape formatting programs were written during the first six months of the study and several types of data entered into the data base file. During the remainder of the one year contract period experiments will be conducted on phantoms, animals, and human subjects to acquire the necessary additional data described in the contract.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 11/19/74; 3/30/76
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $58,126

CONTRACT RESEARCH SUMMARY

Title: Fabrication and Evalution of Computerized Transaxial X-Ray Reconstruction System

Principal Investigator: Sadek K. Hilal, M.D., Ph.D.
Name/Address Presbyterian Hospital
Performing Organization: Columbia-Presbyterian Medical Center
New York, New York 10032

Contract Number: NO1-CB-53974
Starting Date: 6/30/75 Expiration Date: 6/29/77

Goal: To establish a new radiologic process that will contribute toward earlier and more accurate detection, identification and localization of cancer pathology in the chest, abdomen, and other anatomical regions, with potential low risk to the patient.

Approach: This study involves two cooperative contracts (NO1-CB-53858 and NO1-CB-53974). In the initial year of the study, equipment and displays will be developed and fabricated for computerized transaxial x-ray reconstruction for parts of the torso analogous to present equipment used for such studies of the head. At the same time, suitable algorithms for radiologic cross-section images of high spatial densitometric and temporal resolution will also be developed. In the ensuing two year endeavor, clinical trials of the equipment will be pursued on suitable human subjects and results compared with conventional diagnostic radiological and clinical examinations taken on the same subjects. Instrumentation and algorithms modifications suggested by engineering evaluations and clinical experience will be incorporated as warranted and feasible. Independent appraisal of the radiographic results and diagnostic success will be made by a panel of experts selected by the National Cancer Institute.

Progress: Algorithm evaluations have been carried out to test (1) detector fan of rays instead of a source fan; (2) tradeoffs in resolution between number of views and number of readings per view; (3) effect of polychromaticity on image quality; (4) production of lateral and frontal reconstructions; (5) filters, using patient data; (6) effect of decentering the body scanner. A protocol is being evolved for clinical evaluation of the body scanner when it becomes available. The evaluation protocol will be subdivided into body regions with separate physicians directly responsible for each region.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 11/20/74; 5/5/76
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $199,290

CONTRACT RESEARCH SUMMARY

Title: Algorithms for Computerized Transaxial X-Ray Reconstruction

Principal Investigator: Gabor T. Herman, Ph.D.
Name/Address Research Foundation of SUNY
Performing Organization: Albany, New York 12224

Contract Number: NO1-CB-53860
Starting Date: 6/30/75 Expiration Date: 6/29/76

Goal: To improve detection and diagnosis of early cancer lesions by means of improved radiologic imaging techniques involving new mathematical algorithms for computerized transaxial x-ray reconstruction.

Approach: Algorithms will be developed for computerized three-dimensional reconstruction of x-ray absorption techniques from projection data collected via various sources and detector geometries. The reconstruction will minimize the anatomical and densitometric distortion and artifactual effects in regions of medical interest and decrease both scan time and radiation risk of the patient. Criteria also will be developed for evaluating efficacy of the algorithms. The algorithms will be written in a high level programming language such as FORTRAN and assembled in a library which takes advantage of input and output commonalities and is modular and expandable. The library of algorithms plus suitable mathematical and actual test data and results will be written on nine track industry compatible tape and delivered to the National Cancer Institute for distribution to other investigators in the field.

Progress: An algorithm has been devised, implemented and tested which can be used in a scanning device requiring less than 10 seconds scan time to detect lesions of 1 cm diameter or less in the chest or abdomen. Data base creation currently is in progress. A realistic detailed phantom of the human thorax has been produced along with simulated polychromatic x-ray projections for it. Subroutines have been written for most of the algorithms to be incorporated into the programming system and theoretical groundwork has been established for comparative evaluation of algorithms. Work also is progressing on the required display program.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 11/19/74; 3/30/76
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $54,402

CONTRACT RESEARCH SUMMARY

Title: Algorithms for Computerized Transaxial Nuclide Reconstruction

Principal Investigator: T. F. Budinger, M.D., Ph.D.
Name/Address ERDA - Lawrence Berkeley Laboratory
Performing Organization: University of California, Berkeley
Berkeley, California 94720

Contract Number: Y01-CB-50304
Starting Date: 6/30/75 Expiration Date: 6/29/77

Goal: To improve detection and diagnosis of early cancer lesions using radioactive compounds.

Approach: Hardware and software for computerized transaxial nuclide reconstruction based on transverse section emission data from human subjects is in current development in numerous institutions. Such procedures appear particularly promising for use with structure-specific labeling with a wide array of radioactive compounds. Emission imaging may permit not only static reconstruction but also quantitative estimates of time-course movements of labeled compounds through structures located deep within the body. Transaxial reconstruction will permit precise estimates of location of radioactivity in small body volumes that would be obscured by surrounding radioactivity and internal radioabsorption in conventional viewing techniques. In the present project algorithms will be developed for computerized reconstruction of clinical data produced by established and newly developed transaxial nuclide projection machines. In addition, techniques for algorithm evaluation and comparison will be developed and applied on suitable materials. Algorithms, written in a high level programming language such as FORTRAN, together with phantom, animal and human emission and transmission projection data and reconstructions will be recorded on nine track industry compatible magnetic tape and delivered to the National Cancer Institute. The National Cancer Institute shall make independent evaluations and disseminate this information to other investigators.

Progress: The program library and user's manual is comprised of methods for quantitatively depicting the three dimensional distribution of radiopharmaceuticals in the body. Algorithms include iterative least squares methods (3), ART (2), SIRT (2), backprojection of filtered projections (multiple filters). and filtering the backprojection (multiple filters). The library includes 4 methods of weighting backprojections, and 8 techniques of compensating for attenuation. Studies have been done on phantoms and patients using these algorithms and include quantitative evaluation of brain and liver tumors and change in tumor volume with therapy. Two schemes of hard-wiring reconstruction algorithms are being developed to provide widespread usefulness of those techniques found to be suitable from experience with the above large accumulation of reconstruction strategies.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 3/31/76
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $91,089

CONTRACT RESEARCH SUMMARY

Title: Exploration of the Use of a Proton Beam in Tissue Densitometry

Principal Investigators: Cornelius A. Tobias, Ph.D., and Eugene V. Benton, Ph.D.
Name/Address ERDA - Lawrence Berkeley Laboratory
Performing Organization: University of California, Berkeley
Berkeley, California 94720

Contract Number: Y01-CB-40302
Starting Date: 6/30/74 Expiration Date: 6/29/77

Goal: To evaluate the diagnostic potential of accelerated proton and various heavy ion beams in the detection of tissue abnormalities poorly or not detectable with x-rays; to develop techniques for the use of the particle beam of choice as a low dose adjunct to x-ray diagnostics.

Approach: With suitable phantoms and *in vivo* and *in vitro* biological specimens, the resolution and density detection sensitivity of various accelerated charged particle beams (H, He, C, O, Ne) will be compared using the LBL Bevatron/Bevalac for $Z \geq 2$ beams and the Harvard cyclotron for proton beams. In addition to a variety of particle types, the importance of beam energy and suitability of various detectors (such as plastic nuclear track detectors and photographic film) will be evaluated for the optimum beam particle-energy-detector system. Human diagnostic studies in cancer detection with volunteer patients will be conducted with the system of choice. Comparisons of particle radiography with other non-invasive diagnostic techniques and pathology (when possible) will be made. An initial patient mammographic study with a few select patients will be conducted to develop particle radiographic techniques. A larger sampling will follow (with the same aforementioned comparisons) to accumulate sufficient statistics for diagnostic evaluation of particle mammography. Concurrently initial particle radiographic studies in the diagnosis of soft-tissue abnormalities of the brain and skeletal abnormalities will be made.

Progress: The studies with phantoms to date indicate that heavy ion beams (C, O, Ne) using stacks of plastic foils for image registration afford substantially better resolution (lateral and depth) than proton beams using film stacks for track registration, even when the proton dose is much larger than the heavy ion dose, to the phantom. The heavy ion/plastics system produces better depth resolution than a conventional x-ray/film system. Limited studies with freshly excised breast specimens suggest the same conclusions as the phantom studies. A technique for patient carbon mammography has been developed and six patients radiographed with promising results. Further particle/detector comparative work and patient particle radiography of various organs is planned.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Dates: 1/20/75; 3/23/76
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $102,622

CONTRACT RESEARCH SUMMARY

Title: Exploration of the Use of a Proton Beam in Tissue Densitometry

Principal Investigator: John F. Mullan, M.D.
Name/Address University of Chicago
Performing Organization: Chicago, Illinois 60637

Contract Number: NO1-CB-43918
Starting Date: 6/30/74 Expiration Date: 6/29/76

Goal: To determine if a proton beam from a high energy (200-400 Mev) source is capable of improving the diagnosis of cancer and reducing the dosage of ionization radiology.

Approach: While it is believed that the theoretical studies made and the experimental results already obtained indicate that proton radiography may have great potential as a medical diagnostic tool, it is hoped in the present study to demonstrate this fact conclusively. This work will include physical measurements on test objects to define quantitatively the capabilities and limitations of proton radiography. Measurements on tissue specimens will be carried out and the results also will be compared with density measurements in a density column.

Progress: Brief exploratory studies have been made to determine factors to be considered in developing a properly shielded proton beam suitable for clinical diagnostic use. Fewer than expected results were obtained in this study in part because of the unavailability to the study of proton beams at Argonne National Laboratory. Since the unavailability continued during the second, and final, year of the study, work has been concentrated on attempts to correlate densities, determined by gradient column measurements, with proton beam densities measured at Harvard University.

Significance of Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 1/22/75
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $84,079

CONTRACT RESEARCH SUMMARY

Title: Algorithms for Computerized Transaxial Nuclide Reconstruction

Principal Investigator: David E. Kuhl, M.D.
Name/Address University of Pennsylvania
Performing Organization: Philadelphia, Pennsylvania 19174

Contract Number: NO1-CB-53859
Starting Date: 6/30/75 Expiration Date: 6/29/77

Goal: To improve detection and diagnosis of early cancer lesions using radioactive compounds.

Approach: Hardware and software for computerized transaxial nuclide reconstruction based on transverse section emission data from human subjects is in current development in numerous institutions. Such procedures appear particularly promising for use with structure-specific labeling with a wide array of radioactive compounds. Emission imaging permits not only static reconstruction but also quantitative estimates of time-course movements of labeled compounds through structures located deep within the body. Transaxial reconstruction will permit precise estimates of location of radioactivity in small body volumes that would be lost or obscured by surrounding radioactivity and internal radioabsorption in conventional viewing techniques. In the present project, algorithms will be developed for emission circumferential aperture reconstruction tomography (ECART) from clinical projection data collected by a new transaxial coded ring aperture radionuclide scanner with high count efficiency.

Progress: Four different encoding mask systems for performing radionuclide emission circumferential aperture reconstruction tomography (ECART) in an axial plane have been modeled and their performance characteristics analyzed. The four mask systems include the pinhole camera, a noise-like rotating mask, a multiple gear-mask system and a time-modulated aperture system. The systems have been compared by evaluating their total counting efficiencies and their signal-to-noise limits. An optimal ECART has been found to detect many more photons per counting period than detected by a conventional collimated detector system; however, for distributed radiation sources both systems have approximately equal signal-to-noise ratios. ECART also has shown greater flexibility than collimated detector systems for optimally reconstructing objects of different sizes.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 3/31/76
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 75 Funds: $71,825

CYTOLOGY AUTOMATION CONTRACT PROGRAM

The Committee on Cytology Automation was established in July, 1972 to advise the Director, Division of Cancer Biology and Diagnosis, National Cancer Institute on all matters pertaining to Cytology Automation. Specifically, the Committee (1) serves as advisors to National Cancer Institute on the state of the art in scientific and technical areas which bear on automation, and (2) advises the National Cancer Institute on plans and implementation in operational terms for National Cancer Institute's contractual support of developing automation in cytopathology.

The Committee determined that six areas are within the mission of the Committee:

1. Specimen Collection
2. Specimen Preparation
3. Sensing-screening Systems
4. Decision-diagnostic Systems
5. Specimen Sources
6. System Evaluation

At the present time several very important areas of research have been accomplished. In area 1, specimen collection, it has been determined in an extensive study by Dr. Marluce Bibbo and collaborators at the University of Chicago that the number of single abnormal cells present in gynecologic apecimens is adequate for screening purposes in essentially all cases of pre-malignant and malignant lesions. Thus only relatively gentle dis-aggregation procedures are necessary in preparing clinical specimens for automated analysis.

In area 2, specimen preparation, a thorough examination of mechanical and enzymatic dispersal procedures to increase the yield of single intact cells has been completed by several contractors. The results of this work indicate that relatively simple mechanical dispersal techniques such as pressure-controlled syringing and ultrasonic treatment produce adequate numbers of morphologically and cytochemically intact cells for instrumental analysis.

In areas 3, 4, and 6, development and evaluation of integrated screening systems, several automated analytic systems have been identified as promising potential designs for automated clinical prescreening systems. Evaluation and development of these systems is proceeding to, a) ensure that they reflect a sound biologic basis for identification of pre-malignant and malignant squamous cell lesions of the gynecologic tract; b) evaluate their performance characteristics, especially preliminary analysis of sensitivity and specificity.

Work is continuing on the identification of quantitative descriptors of pre-malignant and malignant lesions, especially cytochemical and immunological markers which will increase the potential sensitivity and specificity of currently employed sensor sytems in screening clinical material.

CONTRACT RESEARCH SUMMARY

Title: Evaluation of ^{67}Ga for the Automated Detection of Neoplasia of the Uterine Cervix

Principal Investigator: Carolus Cobb, Ph.D.
Name/Address Performing Organization: American Science and Engineering, Inc.
955 Massachusetts Avenue
Cambridge, Massachusetts 02139

Contract Number: NO1-CB-53931
Starting Date: 6/30/75 Expiration Date: 3/29/76

Goal: Determination of the cell types from human uterine cervix showing an increase affinity for ^{67}Ga.

Approach: The contractor will study cells prepared with the AS&E technique for inducing differential uptake for a possible use of ^{67}Ga as a marker for cytology automation. Cell types investigated will be normal, pre-malignant and malignant human vaginal-cervical cells. Study of sub-cellular localization of the increased ^{67}Ga uptake will also be performed.

Progress: Techniques for innoculation of cells on slides, for induction of differential uptake of ^{67}Ga, and for autoradiography have been developed. Data from normal and abnormal exfoliated cells are being acquired.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
Investigation of differential uptake of ^{67}Ga for a possible use of ^{67}Ga as a marker for distinguishing neoplastic from non-neoplastic human cells.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 6/24/74
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $49,633

CONTRACT RESEARCH SUMMARY

Title: Application of Flow System Methods of Cell Analysis and Sorting to Cancer Screening

Principal Investigator: M. Ingram, M.D.
Name/Address Performing Organization: ERDA-Los Alamos Scientific Laboratory
Los Alamos, New Mexico 87115

Contract Number: NO1-CB-60311
Starting Date: 4/1/71 Expiration Date: 3/31/77

Goal: Automation of clinical cytology screening and diagnosis.

Approach: A multiparameter sorter has been developed and is being tested by the contractor to measure and analyze cytobiological parameters related to malignancy. With both model tumor lines and human squamous specimens, this instrument has separated successfully samples which have as few as 8% tumor cells on the basis of DNA content alone. The goals of the present project are to evaluate a) the PI-FITC two color fluorescence system b) the time-of-flight sensors in a dual parameter mode and c) the multi-angle light scatter sensor (all of which were developed at LASL under previous NCI contracts) as to their usefulness in gynecologic cancer screening. Also under subcontract from LASL, Grumman Aerospace Corporation will investigate the applicability of Fourier transform analytic techniques to screening of clinical vaginal-cervical cytology. It is also proposed that algorithms will be designed, tested, and implemented for strict quantitative description of the parameters to be evaluated.

Progress: The contractor has obtained a large number of gynecologic specimens for which some dispersal, fixation, and staining techniques have been developed. The acriflavin-feulgen procedure for DNA staining, mithramycin as a rapid DNA stain, propidium iodide and fluorescein isothiocyanate as a simultaneous DNA-protein staining technique, narrow angle light scatter, multi-angle light scatter, time-of-flight, and Coulter volume parameters have been successfully implemented on flow systems at LASL under NCI contracts. These all show high potential as useful parameters in gynecologic cancer screening. The contractor has also made major improvements in the electronic cell sorting technology which will be used to demonstrate the correlation between these parameters and the morphology.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
The use of a very rapid zero-resolution automated screening system in clinical cytodiagnosis will allow greater availability of these services to a much larger population with improved diagnostic accuracy.

Project Officer: B. J. Fowlkes
Program: Cytology Automation Site Visit Date: 2/3/76, 1/7/76
Technical Review Group: Cytology Automation Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $348,200

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Markers for Gynecologic Specimens

Principal Investigator: Professor Torbjorn Caspersson, M.D.
Name/Address Karolinska Institute
Performing Stockholm, Sweden
Organization:

Contract Number: NO1-CB-43945
Starting Date: 6/30/74 Expiration Date: 6/29/77

Goal: Automation of clinical cytologic screening and diagnosis, concomitant with improvement in diagnostic accuracy and throughput.

Approach: Dr. Caspersson has pioneered the development of innovative methods of investigative cytology and cytogenetics. Under this contract, he will develop and evaluate prototype machines for automatically measuring cytologic features of clinical gynecologic specimens using high resolution scanning microscopy with absorption ultraviolet-spectrophotometry, interferometry and cytofluorometry, individually and in combination. Quantitative measurements of the various cell types of the female genital tract will yield multiparameter data which can be used as discriminators for these different cell types by automated cytology instruments.

Progress: A two parameter instrument has been constructed. The instrument consists of computer controlled servomotor driven scanning microscope, microspectrophotometer and microinterferometer. The scanning microscope is used to examine and select cells and has the capability to recall the previously chosen cells for reexamination and analysis. The integrated instrument permits microspectrophotometric measurements in the UV and/or visible range, followed by microinterferometric measurements on the same cell while the cell remains in the same microscope field of vision. Modification and improvement are being made including incorporation of image intensifier - camera system.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
Development of very rapid high resolution multiple modality automated scanning microscopes will advance image processing approaches for cytologic screening and diagnosis, and promote better understanding of normal, precancerous and cancerous states.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 2/27/74
Technical Review Group: Committee for Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $35,000

CONTRACT RESEARCH SUMMARY

Title: New Stains and Other Optical Markers Useful for Gynecologic Specimens

Principal Investigator: Brian H. Mayall, M.D.
Name/Address Performing Organization: Lawrence Livermore Laboratory
Livermore, California 94550

Contract Number: Y01-CB-40300
Starting Date: 6/1/74 Expiration Date: 6/29/76

Goal: Development and preliminary clinical testing of stains useful in differentiating normal from non-normal cytopathological material.

Approach: Utilizing their unique combination of flow-sorting and image-processing expertise, the investigators will evaluate specimen preparation and staining techniques on clinical samples. The ability to evaluate specimens at both zero and medium to high resolution will assure the mutual compatibility of preparative techniques and safeguard the diagnostic value of samples prepared for automated systems.

Progress: Clinical specimens have been collected and used for production of monodisperse cell suspensions. The cells are treated by mechanical means, e.g., syringing and chemicals, and by enzymes and toxins. Cells of various types have been photographed and mapped for quantitative cytochemistry. The approach ensures that specific cell types are analyzed as the CYDAC is employed to digitize and compile data on these cells. Correlation between data and selected cells can be made since each cell was previously photographed and recorded. Cells have been stained with toluidine blue, naphthol yellow S and alkaline fast green. Cells in suspension are also quantitatively analyzed by the LLL flow microfluorometer. Data have been obtained pertaining to different fluorescent properties of the various cell types. More samples are being analyzed. Light scattering properties of cells are also being explored.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
Development and evaluation of techniques to rapidly screen cell populations for pre-malignant and malignant cells.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 2/1/74 - 10/31/74
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $314,740

CONTRACT RESEARCH SUMMARY

Title: High-Speed Flow System Methods of Cell Analysis and Sorting

Principal Investigator: M. Ingram, M.D.
Name/Address Performing Organization: ERDA-Los Alamos Scientific Laboratory
Los Alamos, New Mexico 87115

Contract Number: Y01-CB-10055
Starting Date: 4/1/71 Expiration Date: 3/31/76

Goal: Automation of clinical cytologic screening and diagnosis.

Approach: A multiparameter sorter has been developed and is being tested by the contractor to measure and analyze cytobiological parameters related to malignancy. With both model tumor lines and human squamous specimens, this instrument has separated successfully samples which have as few as 8% tumor cells on the basis of DNA content alone. The goals of the present project are biomedical rather than bioengineering. The present effort centers specifically on the development of parameters or combinations of parameters which characterize malignancy, development of new sample preparation and staining techniques suitable for analysis by the sorters, the investigation of lectin binding to surfaces of normal and abnormal squamous cells, the investigation of light scattering and time-of-flight properties of squamous cells.

Progress: The major biological thrusts of the program for the past year include application of the acriflavin - Feulgen procedure as a DNA stain for cervical-vaginal cells, the use of 9-hydrazine acridine as a specific marker for aldehyde groups on DNA, evaluation of acridine orange as a rapid stain, development of mithramycin as a rapid DNA stain, continued evaluation of the collagenase dispersal procedure, and continued development of the DNA-protein staining technique. Instrumentation development has included improved light-scatter signal resolution and a LED strobe Lamp-driver unit for the cell sorters; construction of a prototype flat flow chamber, a time-to-amplitude converter, a dual-channel logarithmic amplifier, and the development of a 32-element circular ring photodiode array with associated fast log amplifiers and computer interface, computer-controlled analysis and cell sorting capability, and electronics for cell doublet discrimination. The contractor has evaluated over 860 gynecologic specimens during the past year using techniques developed at LASL.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
The use of a very rapid zero-resolution automated screening system in clinical cytodiagnosis will allow greater availability of these services to a much larger population with improved diagnostic accuracy.

Project Officer: B. J. Fowlkes
Program: Cytology Automation Site Visit Date: 1/18/73 - 6/14/74
Technical Review Group: Cytology Automation Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $348,200

CONTRACT RESEARCH SUMMARY

Title: Development of Hematoxylin Substitutes for Staining of Tissues and Exfoliated Cells

Principal Investigator: Ralph D. Lillie, Ph.D.
Name/Address Louisiana State University
Performing Baton Rouge, Louisiana 70586
Organization:

Contract Number: NO1-CB-43912
Starting Date: 1/11/76 Expiration Date: 1/10/79

Goal: Development of hematoxylin substitutes for diagnostic staining of tissues and exfoliative cells.

Approach: 1. Synthesize various classes of mordant dyes or obtain same from either specific manufacturers or from the archives of the Biological Stain Commission. 2. Perform studies on various tissues with combinations of dyes obtained to achieve staining in accordance with criteria satisfactory to the Project Officer and to the Biological Stain Commission's certification criteria. 3. Procure samples of possibly suitable mordant dyes offering blue or black mordant colors which may be suitable to replace hematoxylin in one or more of its histological staining uses. 4. Subject such samples to the usual tests applied by the Biological Stain Commission for the certification of hematoxylin. 5. Apply favorable test results to routine surgical diagnostic material, in Papanicolaou smear diagnosis, and in other staining procedures applicable to the differential diagnosis of various cancers, evaluating results in each case in comparison of previously standard hematoxylin procedures and with the best of the previously proposed substitutes.

Progress: Synthesis and testing are on schedule as outlined above.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
Stain development is essential to the cytological diagnosis of cancer.

Project Officer: M. F. Stanton, M.D.
Program: Cytology Automation Site Visit Date:
Technical Review Group: Ad Hoc Committee Lab. Pathology
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $46,362

CONTRACT RESEARCH SUMMARY

Title: Methods for Obtaining Monodisperse Suspensions of Cytology Specimens

Principal Investigator:
Name/Address
Performing
Organization:

Leopold G. Koss, M.D.
Montefiore Hospital and Medical Center
Bronx, New York 10467

Contract Number: NO1-CB-43963
Starting Date: 6/1/74 Expiration Date: 5/31/76

Goal: To obtain monodisperse cell suspensions from gynecologic cytopathology specimens for use in flow-through systems being developed for cytology automation.

Approach: The principal investigator will study the natural cell attachments and various ways of disrupting these attachments. Electron microscopy will be used to evaluate the effectiveness of the cell attachment (chiefly desmosome) disruption.

Progress: Vaginal pool aspirates from patients ranging in age from 19 to 74 have been studied electron microscopically to examine the cell adhesions. Extensive interdigitations and typical desmosomes were seen in the squamous cells. Several desmosome-forming culture lines are being studied with electron microscopy. Cell samples from the female genital tract either suspended in normal saline or fixed in 1% glutaraldehyde were treated with the following dispersing agents: trypsin (0.25%), EDTA (0.1%), collagenase (0.25%) and sodium desoxycholate (1%). The dispersing agents had no effect on the glutaraldehyde-fixed samples and the dispersal of cell clumps was inadequate in the saline suspension samples. Studies are being conducted using syringing alone or in combination with chemical treatment as possible disaggregation methods. Studies are underway to isolate desmosomes from gynecologic tissue in order to perform studies of various biochemical and enzymatic agents on the desmosomes.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Mary Cassidy
Program: Cytology Automation Site Visit Date: 2/7/74, 11/11/74 &
Technical Review Group: Ad Hoc Committee 11/18/75
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $180,000

CONTRACT RESEARCH SUMMARY

Title: Furnishing a Cytoscreener and Technician for Testing Purposes

Principal Investigator: Melvin P. Ehrlich
Name/Address Performing Organization: Nuclear Leasing Corporation
P.O. Box 3511
Hyde Park, New York 11040

Contract Number: NO1-CB-53915
Starting Date: 6/1/75 Expiration Date: 7/31/75

Goal: To supply Cytoscreener for engineering and biological evaluation.

Approach: The Cytoscreener, manufactured and patented by Nuclear Research Associates, has been proposed as an instrument suitable for automation of clinical cytologic screening. Clinical tests have been equivocal. Basic engineering and biologic data are needed before further clinical testing can be conducted.

Progress: Cytoscreener has been delivered and is undergoing analysis by Utah Biomedical Test Laboratory.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 10/11/74
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $113,618

CONTRACT RESEARCH SUMMARY

Title: New Stains and Other Optical Markers for Clinical Specimens in Suspension

Principal Investigator:
Name/Address
Performing
Organization:

Robert C. Leif, Ph.D.
Papanicolaou Cancer Research Institute
Miami, Florida 33123

Contract Number: NO1-CB-43962
Starting Date: 6/30/74 Expiration Date: 6/29/76

Goal: Investigation of new markers and techniques of specimen preparation for clinical cytopathologic screening.

Approach: The investigator will extend the technique of centrifugal cytology to produce improved specimens for analytic instrumentation. Techniques for dialysis staining, heavy metal chelating fluorochromes and immunofluorescence will be investigated as preliminary feasibility studies.

Progress: Studies are in progress using centrifugal cytology to monitor dissociation of cells previously treated by various chemicals. However, much testing is to be done and combination of other chemicals may be necessary. Improved centrifugal cytology buckets has been constructed. These should significantly aid in monitoring cell dissociation and immunofluorescent testing. Antibodies have been prepared against HSV-2 and also against cervical carcinoma antigen. The antibodies will be used as possible markers for abnormal cells. Specificity of CCA is being tested using gridded slides for cell-by-cell analysis. A europium chelate with a narrow band emission spectrum and a primary amino group has been synthesized. Several β-diketonato complexes have been characterized as to solubility and emission spectra. These fluorochromes will be explored for use in automated instruments in detecting various cell types.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
The development of new markers and preparative techniques for clinical specimens will improve the prospects for more rapid automation of clinical cytopathologic pre-screening.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 3/11/74 - 12/20/74
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $150,000

CONTRACT RESEARCH SUMMARY

Title: New Methods for Obtaining Monodisperse Cells and Markers for Gynecologic Specimens

Principal Investigator: Paul Todd, Ph.D.
Name/Address Pennsylvania State University
Performing University Park, Pennsylvania 16802
Organization:

Contract Number: NO1-CB-43984
Starting Date: 6/30/74 Expiration Date: 6/29/76

Goal: Investigation of new techniques for preparing and marking cells for automated pre-screening of clinical cytology specimens.

Approach: Cell electrophoresis, both micro and bulk, will be investigated. Heavy meromyosin binding to α-filaments in normal cells of the gynecologic tract will be investigated. Potential utility of HSV II antigens in distinguishing normal from abnormal human cells will be explored. A number of enzymatic techniques for cell dispersal will be investigated and analyzed.

Progress: Heavy meromyosin has been prepared and purified from chicken breast muscle. Testing of meromyosin binding is in progress. Animal model systems indicate that malignant cell surface changes are such that there is surface binding. Human clinical specimens are being tested. Antibodies against the heavy fragment are being prepared to be used as possible markers for abnormal cells. Antisera are collected and used to seek the non-virion antigen, Ag-4. Isolation of HSV non-virion antigen is under way. The antigen - antibody reaction of abnormal cells is another marker being explored. Cell electrophoresis has been applied to many cell types including virus-transformed cells. Results indicate that cell surface alterations are sufficient to influence the cell migration rates. Cell electrophoresis is being used to differentiate normal and abnormal cells of the female genital tract. Many enzymes have been applied to dissociate cells. Enzymes are used one at a time or in combination of a few enzymes. Enzyme analysis and purification is also being done to ensure the qualitative analysis. No single enzyme has been found to effectively dissociate cells.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
Cell preparative techniques and exploitable markers hold the key to automation of cytology screening.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 4/4/74 - 10/24/74
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $216,841

CONTRACT RESEARCH SUMMARY

Title: Markers for Detection of Abnormal Cervical Cells by Optical Methods

Principal Investigator: B. D. Halpern, Ph.D.
Name/Address Polysciences, Inc.
Performing Warrington, Pennsylvania 18976
Organization:

Contract Number: NO1-CB-43943
Starting Date: 6/1/74 Expiration Date: 5/31/76

Goal: Chemical synthesis and preliminary testing of markers for clinical specimens.

Approach: The Contractor will apply his extensive experience in biochemical synthesis to produce absorptive and flourescent dyes to stoichiometrically quantitate macromolecules, enzymatic activity and antigen-antibody systems suggested by basic scientific research to be of potential utility in distinguishing human malignant and pre-malignant cells from non-neoplastic cells in automated cytopathologic screening instruments.

Progress: Fluorescent labeled microspheres have been synthesized. Specific antibodies from other contractors are being conjugated to these spheres for possible use as markers for abnormal cells in the direct or indirect immunofluorescent test. Specially coated glass slides have been made. The slides are capable of binding γ-globulin and will be used for abnormal cell enrichment (on slides) by interaction of these binding sites and antibody - conjugated abnormal cells. Many DNA - and protein - specific fluorochromes are purified for use by other contractors. Synthesis of several new fluorescent macromolecular stains has begun, with specificity oriented toward RNA species and certain related molecules, such as RNA polymerase. These stains are to be used with other well-defined stains to acquire multi-parameter quantitative and qualitative measurements to differentiate normal and abnormal cells by instruments now operational at other contractors' laboratories.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
Chemical synthesis and verification of stoichiometric stains useful for clinical use in screening cytopathology specimens.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 1/25/74 - 10/25/74
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $139,896

CONTRACT RESEARCH SUMMARY

Title: Vaginal and Cervical Cell Sample Sources for Cytology Automation

Principal Investigator: Walter E. Tolles, Ph.D.
Name/Address SUNY - Downstate Medical Center
Performing Brooklyn, New York 11203
Organization:

Contract Number: NO1-CB-53929
Starting Date: 6/30/75 Expiration Date: 6/29/76

Goal: Evaluation of multiparameter cell sorter designed for cytology automation.

Approach: The contractor shall deliver to the Laboratory of Pathology 775 gynecologic cytopathology samples a year obtained according to a protocol supplied by NCI. These samples will range in diagnoses from normal to squamous cell carcinoma and adenocarcinoma. They will be used to evaluate the multiparameter cell sorter and methods of staining and cell dispersal being investigated for eventual use in a fully automated clinical instrument.

Progress: The contractor has delivered to NCI gynecologies cytology samples which have been used in a number of studies. A portion of these samples has been sent to other contractors for use in their studies.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)
The accessibility of gynecologic cytopathology samples of all types of abnormality will expedite the evaluation and experimentation in the program of automated cytology.

Project Officer: Mary Cassidy
Program: Cytology Automation Site Visit Date: 11/19/75
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $25,530

CONTRACT RESEARCH SUMMARY

Title: Methods for Obtaining Monodisperse Preparations of Cytology Specimens

Principal Investigator: Walter E. Tolles, Ph.D.
Name/Address SUNY - Downstate Medical Center
Performing Brooklyn, New York 11203
Organization:

Contract Number: NO1-CB-43955
Starting Date: 6/1/74 Expiration Date: 5/31/76

Goal: To obtain a monodisperse cell sample from gynecologic cytologic specimens for use in flow-through and image processing systems being developed for cytology automation.

Approach: The emphasis will be on lytic and enrichment techniques. Chemical and enzymatic agents as well as mechanical and ultrasonic shearing techniques will be used to break up clusters. Rate-zonal sedimentation will be used as an enrichment technique.

Progress: An automatic syringer has been fabricated and analysis of cell disaggregation and cell damage has been accomplished in five cervical specimens. Collagenase treatment of cervical samples has been attempted and best results obtained from unfixed samples suspended in saline. Rate zonal sedimentations have been made on fifty cervical samples and the distributions of cell types in the various fractions has been quantified. Ultrasound has been studied as a possible shearing technique and the results appear promising.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Mary Cassidy
Program: Cytology Automation Site Visit Date: 2/8/74-11/12/74-11/19/75
Technical Review Group: Ad Hoc Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $82,677

CONTRACT RESEARCH SUMMARY

Title: Vaginal and Cervical Cell Sample Sources for Cytology Automation

Principal Investigators: Mr. Arthur Hontz/Earl Greenwald, M.D.
Name/Address Performing Organization: Temple University Medical School and Hospital
Philadelphia, Pennsylvania 19140

Contract Number: NO1-CB-43937
Starting Date: 6/30/74 Expiration Date: 6/29/76

Goal: Evaluation of multiparameter cell sorter designed for cytology automation.

Approach: The contractor shall deliver to the Laboratory of Pathology 1000 gynecologic cytopathology samples a year obtained according to a protocol supplied by NCI. These samples will range in diagnoses from normal to squamous cell carcinoma and adenocarcinoma. They will be used to evaluate the multiparameter cell sorter and methods of staining and cell dispersal being investigated for eventual use in a fully automated clinical instrument.

Progress: The contractor is delivering approximately 115 samples per month to the NCI. The range of sample types has been from normal through invasive carcinoma. These samples are being used in sample preparation studies as well as for evaluating NCI's multiparameter cell sorter. A number of these samples have been sent to other contractors for use in their studies.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)
The accessibility of gynecologic cytopathology samples of all types of abnormality will expedite the evaluation and experimentation in the program of automated cytology.

Project Officer: Mary Cassidy
Program: Cytology Automation Site Visit Date: 7/11/74
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $33,742

CONTRACT RESEARCH SUMMARY

Title: Evaluate Stains Useful for Gynecologic Specimens

Principal Investigator: Seymour West, Ph.D.
Name/Address University of Alabama
Performing Birmingham, Alabama 35294
Organization:

Contract Number: NO1-CB-43960
Starting Date: 6/30/74 Expiration Date: 6/29/77

Goal: Evaluate the applicability of biophysical instrumental analysis to clinical cytopathologic pre-screening.

Approach: The contractor will apply biophysical techniques to evaluate acridine orange as a molecular probe and fluorescence fading phenomenon.

Progress: Nuclear fluorescence intensity of fixed cells from clinical gynecologic specimens and Acridine Orange fluorescent fading of nuclear macromolecules have been under investigation.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
Identification of new machine sensible properties suitable for distinguishing neoplastic from non-neoplastic human cells.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 1/22/74,11/22/74
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $299,790

CONTRACT RESEARCH SUMMARY

Title: Investigation of the Diagnostic Value of Gynecologic Cytopathology Samples

Principal Investigator: Marluce Bibbo, M.D., Sc.D.
Name/Address Performing: University of Chicago
Chicago, Illinois 60637

Contract Number: NO1-CB-43919
Starting Date: 6/15/74 Expiration Date: 6/14/76

Goal: To determine the cellular content of cytologic samples from vagina, ectocervix and endocervix of patients with various clinical conditions.

Approach: The principal investigator is using the large volume of Pap smears available at the University of Chicago, which have been collected over the past 18 years, for a retrospective study of the cellular content of the smears. The smears are being examined and counted for relative numbers of epithelial and inflammatory cells of all types from normal through invasive carcinoma cases. It will be noted if cells appear singly or as part of a cluster and if the cells are degenerate or well preserved. The classification will be according to that of the World Health Organization.

Progress: 83 cases have been counted which include 18 normal cases, 10 cases of severe dysplasia, 25 cases of carcinoma in situ 25 cases of poorly differentiated squamous carcinoma, and 5 cases of moderate dysplasia. Quality control studies have been performed to insure reproducibility of results.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)
It is essential in designing an automated screening system to know the content of the samples to be screened.

Project Officer: Mary Cassidy
Program: Cytology Automation Site Visit Date: 2/20/74 - 12/6/74
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $62,500

CONTRACT RESEARCH SUMMARY

Title: Image Processing for Development of Automated Cell Recognition System

Principal Investigator:
Name/Address
Performing
Organization

George L. Wied, M.D.
University of Chicago
Chicago, Illinois 60637

Contract Number: NO1-CB-33873
Starting Date: 5/31/73 Expiration Date: 4/30/76

Goal: Automation of clinical cytologic screening and diagnosis, focusing on definition of information requirements and criteria for high throughput, high resolution image processing-based decision making.

Approach: The contractor shall evaluate information requirements for characterization and recognition of normal, premalignant and malignant cells from the human female genital tract by means of digital image processing computer feature extraction applied to high resolution, multicolor digital images of clinical specimens. The cytologic material will include 15 main categories with at least 100 cells each, from different subjects, so as to include the effects of intra- and inter-individual variation, physiological age, pregnancy, viral infection, and specific and non-specific inflammatory reactions. Robustness of cytologic features and discrimination logic will be assessed as a function of minor variations in staining and fixation procedures.

Progress: Digitized cell images have been compiled. These data are stored on tapes and will be made available to other contractors for further analyses. These cells are from the various cell types encountered in the exfoliated cell specimens from the female genital tract. Image editing system is operational. Stored cleaned cell images will also be made available for other contractors to analyze with their own algorithms. Further details on scanned cells are being done using 3-color scanner. The 3-color data are admitted from the Zeiss Axiomat to the PDP-8i, displayed on the VT05 storage scope for quality control, stored on DEC tape and eventually carried to the PDP-10. More specimens are being scanned and data stored. The 3-color scanner should enrich the data base for cell image processing.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
A rapid, high-resolution, multi-spectral digital scanner for clinical gynecologic screening and diagnosis will promote better health care delivery and therapy monitoring for the populations at large and at high risk.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 11/20/72
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $150,469

CONTRACT RESEARCH SUMMARY

Title: Slit-Scan Technique as an Automated Cancer Prescreening System in Cytology

Principal Investigator: Leon L. Wheeless, Jr., Ph.D.
Name/Address University of Rochester
Performing Rochester, New York 14642
Organization:

Contract Number: NO1-CB-33862
Starting Date: 3/19/74 Expiration Date: 3/18/77

Goal: Automation of clinical cytologic screening and diagnosis.

Approach: The contractor will conduct a preliminary evaluation of the slit-scan flow system as it now exists using normal and abnormal gynecologic cytology samples. False positive and false negative rates will be ascertained as well as the percent of analyzable contours and the proportion of all particles in the sample which are actually analyzed. Any false contours which arise will be recalled and specific hypotheses developed as to their possible origin.

Progress: The studies of sample preparation and slit-scan cell recognition have reached a stage where an evaluation of the slit-scan flow system is necessary in order to determine what further instrument modifications are necessary to make the slit-scan flow system a useful prescreening clinical instrument.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)
The development of a flow-through screening system for cervical cytology will allow greater accuracy, speed and availability in screening and diagnosis of cancerous and precancerous lesions.

Project Officer: Mary Cassidy
Program: Cytology Automation Site Visit Date: 11/8/72, 6/23/75
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $318,244

CONTRACT RESEARCH SUMMARY

Title: High Resolution Fluorescence Measurements and Quantitative Study of Fluorochromes

Principal Investigator:
Name/Address
Performing
Organization:

Leon L. Wheeless, Jr., Ph.D.
University of Rochester
Rochester, New York 14642

Contract Number: NO1-CB-53930
Starting Date: 6/30/75 Expiration Date: 6/29/76

Goal: Automation of clinical cytologic screening and diagnosis

Approach: The Principal Investigator will investigate "high resolution" fluorescence measurements on cells from the human female genital tract. A quantitative study of Acridine Orange and other fluorochromes useful in automated cell analysis systems will be performed.

Progress: Computer programs have been written to facilitate high resolution data collection. Measurements on normal and abnormal gynecologic epithelial cells are being made using various slit sizes. Preliminary spot scan studies have begun.

Significance for Cancer Researchd (NCP Objective 5 Approach 5.4)

Project Officer: Mary Cassidy
Program: Cytology Automation Site Visit Date: 6/23/75
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
Fy76 Funds: $186,000

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Engineering and Biological Operations of a NLC Cytoscreener

Principal Investigators: L. A. Couvillon/Doran R. Klingler, Ph.D.
Name/Address Utah Biomedical Test Laboratory
Performing 520 Wakara Way
Organization: Salt Lake City, Utah 84111

Contract Number: NO1-CB-53867
Starting Date: 6/1/75 Expiration Date: 6/29/76

Goal: Engineering and biological evaluation of the operation of the Cytoscreener.

Approach: The Cytoscreener has been proposed as an instrument suitable for automation of clinical cytologic screening. Clinical tests of the instrument have been equivocal. Basic engineering and biologic data are needed before further clinical testing can be conducted.

Progress: Two major subsystems of the Cytoscreener, imaging and pattern recognition, are being analyzed. Of the imaging system, optics analysis has been completed. Flow system analysis, power supply tests and computer interface have also been concluded. The overall performance of the Cytoscreener will later be evaluated using specific test samples of known parameters.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 9/30/74
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $111,521

CONTRACT RESEARCH SUMMARY

Title: Pre-Clinical Studies of Chemotherapeutic Studies for Mycosis Fungoides

Principal Investigator: Dr. R. M. Folk
Name/Address Battelle Memorial Institute
Performing Columbus, Ohio
Organization:

Contract Number: NO1-CM-43746
Starting Date: 3/01/75 Expiration Date: 2/29/76

Goal: To apply chemotherapeutic agents developed by DCT for topical treatment of skin cancer.

Approach: To increase the knowledge on the pharmacology of chemotherapeutic agents in topical application, systemic investigation of cutaneous irritation, skin sensitization, phototoxicity and photosensitivity will be carried out.

Progress: Subcontract was just awarded to the University of Mississippi School of Pharmacy. Percutaneous absorption and cutaneous irritation studies will be done first.

Significance for Cancer Research (NCP Objective 6 Approach 2)

Project Officer: Dr. Gary Peck
Program: Biology Support Site Visit Date:
Technical Review Group: Ad Hoc Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $130,000

CONTRACT RESEARCH SUMMARY

Title: Computer Services

Principal Investigator:
Name/Address
Performing
Organization:

Mr. W. Bruce Ramsay
Computer Services Division
National Bureau of Standards
Gaithersburg, Maryland

Contract Number: YO1-CB-80047
Starting Date: 7/01/75

Expiration Date: 6/30/76

Goal: To provide computer facility for mathematical computations related to biological systems modeling carried out in the Laboratory of Theoretical Biology and other groups in DCBD, NCI.

Approach: N/A

Progress: N/A

Significance for Cancer Research (NCP Objective 4 Approach 4)

Project Officer: Dr. Mones Berman
Program: Cancer Biology Support
Technical Review Group: Ad Hoc
Relevance Review Group:
FY76 Funds: $65,000

Site Visit Date: N/A

CONTRACT RESEARCH SUMMARY

Title: Maintain an Animal Holding Facility and Provide Attendant Research Services

Principal Investigator: William M. Rickman, Jr.
Name/Address Performing Organization: Cor Bel Laboratories, Inc.
5708-A Frederick Avenue
Rockville, Maryland 20852

Contract Number: NO1-CB-64024
Starting Date: 11/01/75 Expiration Date: 10/31/76

Goal: Maintain and breed a colony of mice and maintain a rabbit colony as specified by the Project Officer.

Approach: To house, feed, and maintain a colony of mice and rabbits according to the National Research Council Standards; to perform specific technical manipulations as directed by the Project Officer; to maintain a breeding colony of specific mouse strains as required by the Project Officer.

Progress: Performance on the contract has been highly satisfactory. Both the mouse and rabbit colonies have been established and are being maintained according to the National Research Council Standards. Specific mouse strains have been bred and breeding colony has been established to meet the requirements of the Project Officer. The technicians assigned to the project have demonstrated their competence in segregating offspring and in keeping accurate records of the various matings performed.

Significance for Cancer Research: A small animal colony is needed to maintain important NCI intramural research concerned with human neoplasia.

Project Officer: Dr. David H. Sachs
Program: Immunology, Support Site Visit Date: Preaward site visit, 6/05/75
Technical Review Group: Ad Hoc Review Group
Relevance Review Group: DCBD Staff
FY76 Funds: $74,590

CONTRACT RESEARCH SUMMARY

Title: NCI Histocompatibility Typing Center

Principal Investigator: Dr. Frances E. Ward
Name/Address Duke University
Performing Durham, North Carolina 27706
Organization:

Contract Number: NO1-CB-53890
Starting Date: 4/01/76 (Approx.) Expiration Date: 3/30/77

Goal: To study HL-A and other cell surface antigens on normal and neoplastic cells.

Approach: Complement dependent cytotoxicity studies will be performed on human peripheral blood lymphocytes and leukemic cells using antisera and cells provided by the Project Officers. Lymphocyte defined antigens will be investigated by mixed lymphocyte culture tests using DNA synthesis as a measure of detection.

Progress: In the first six months of this new contract, 1452 HLA typings were performed (about 58% of the volume called for in workscope). These typings have been of high quality and include 986 typings of leukemia patients and parents, 197 of melanoma, 12 of osteosarcoma, 3 of established tissue culture lines, and 434 of lung cancer, sprue, dermatitis herpetiformis, manic depressive phychoses and schizophrenia. In addition, 48 sera from melanoma and leukemia patients have been analyzed for antibody to HLA antigens.

Significance for Cancer Research (NCP Objective 3 Approach 5)
Development of methodologies in the identification of isolated cell surface antigens is a necessary prerequisite to the identification of tumor specific associated antigens.

Project Officer: G. Nicholas Rogentine, Jr., M.D., Dean L. Mann, M.D.
Peogram: Immunology, Support Site Visit Date: Waived by memo to
Technical Review Group: Ad Hoc Review Group Dr. Berlin 3/31/75
Relevance Review Group: DCBD Staff
FY76 Funds: $220,000

CONTRACT RESEARCH SUMMARY

Title: Cutaneous Hypersensitivity Antigen Reactions

Principal Investigator: Dr. Ariel Hollinshead
Name/Address Performing Organization: George Washington University
2300 I Street, N.W.
Washington, D. C. 20037

Contract Number: NO1-CB-92176
Starting Date: 6/27/69 Expiration Date: 2/28/76

Goal: To determine whether skin testing for delayed hypersensitivity reactions can be used to measure cellular immunity to tumor-associated antigens. To use the skin test technique as an assay for separation and purification of tumor antigens.

Approach: Extraction and purification of skin reactive antigens from carcinoma of the breast, malignant melanoma, and acute leukemia.

Perform skin tests on patients at various stages of disease, to determine relationship of reactivity to clinical course.

Provide antigens for possible use in in vitro assays of cell-mediated immunity.

Progress: Detailed studies on skin reactive antigens associated with malignant melanoma, carcinoma of the breast and acute leukemia have been done. Two types of skin reactive antigens have been separated in malignant melanoma, including one which seems specific for melanoma. Two distinct antigens have been separated for breast cancer also. Dr. Hollinshead has collaborated with Dr. Wells by providing fetal intestinal skin reactive antigen for testing in a family with familial polyposes. Skin test studies on antigens associated with acute leukemia have also been pursued on a collaborative basis. The contractor has made good progress in partial separation of fractions with different specificities and in establishing specificity of delayed hypersensitivity reactions elicited by her solubilized fractions.

Significance for Cancer Research (NCP Objective 5 Approach 4)
A fundamental problem in tumor immunology is the demonstration of tumor specific antigens in human tumors. This contract provides an opportunity to study delayed skin reactions to antigens.

Project Officer: Mrs. Judith Whalen
Program: Immunology: Immunodiagnosis Site Visit Date: 1/3/74
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY76 Funds: $176,383

CONTRACT RESEARCH SUMMARY

Title: Administrative Support Services

Principal Investigator: Ms. J. Aldridge
Name/Address KAPPA Systems, Inc.
Performing 1501 Wilson Blvd.
Organization: Arlington, Virginia 22209

Contract Number: Npl-CB-53866
Starting Date: 11/01/74 Expiration Date: 10/31/76

Goal: To support administrative staff in preparation scientific meetings and the research components in accomplishing their administrative tasks.

Approach: Current staffing situation places impossible demands on the administrative staff and committee chairmen (presently non-NCI scientists) in carrying out their responsibilities. This project is to support such routine tasks consuming now available manpower.

Progress: Executive secretaries and senior staff members were assisted in organizing scientific meetings of various committees. This made it possible to handle effectively the volume of administrative burden without overstraining available resources. On the intramural research level, again routine administrative and support activities were handled with minimal discription of innovative research.

Significance for Cancer Research (NCP Objective 5 Approach 1-7)

Project Officer: Dr. Ihor J. Masnyk
Program: DCBD Site Visit Date: 9/74
Technical Review Group: DCBD - Ad Hoc Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY76 Funds: $339,797

CONTRACT RESEARCH SUMMARY

Title: Studies of the Immune Response of Mice and Rats to Tumor Antigens

Principal Investigator: Dr. Julie Djeu
Name/Address Litton Bionetics, Inc.
Performing 5510 Nicholson Lane
Organization: Kensington, Maryland 20795

Contract Number: NO1-CB-53889
Starting Date: 4//75 Expiration Date: 4/27/76

Goal: Study of immune responses of mice and rats to tumor-associated antigens. Studies on in vivo resistence to tumors and correlation with in vitro cellular and humoral immune responses.

Approach: The kinetics of the cellular and humoral immune responses to tumor-associated antigens of the following tumors will be studied: tumors induced by Gross and other murine leukemia viruses and murine sarcoma virus; methylcholanthrene-induced tumors. The relationship between the detected antigens and viral gene expression of fetal antigens will be extensively studied. The specificity of each of the detected antigens in both the humoral and cell-mediated immune studies are being intensively studied by inhibition assays. The nature of the cells involved and the mechanism of cytotoxicity in the cell-mediated cytotoxicity assays are also being studied. A major emphasis is being placed on relating in vitro cell-mediated reactivity to in vivo protection against tumor growth by adoptive transfer experiment.

Progress: The main emphasis was placed on study of the characteristics of natural cell-mediated immunity of mice against a variety of tumors. Reactivity was shown against several carcinomas and sarcomas, in addition to lymphomas. Tumors which were susceptible to cytotoxicity in vitro were also found to be affected in vivo by natural cellular immunity. This mechanism has been found to be thymus independent, with higher levels of cytotoxic reactivity in nude mice and in thymectomized mice, and to be radioresistant but sensitive to cyclophosphamide. Adoptive transfer experiments with (C58NT) D_1 in rat lymphoma, demonstrated that T cells and not macrophages, were able to protect against tumor growth.

Significance for Cancer Research (NCP Objective 6 Approach 4) Understanding the relationship of in vitro assays to host resistance to tumors is an essential element in properly using these assays to minitor response to immunotherapy.

Project Officer: Ronald B. Herberman, M.D.
Program: Immunology, Support Site Visit Date: None
Technical Review Group: Ad Hoc Review Committee
Relevance Review Group: Staff
FY76 Funds: $ 18,160 Supplement $210,000 Estimate

CONTRACT RESEARCH SUMMARY

Title: Melanoma Cell Vaccine and *In Vitro* Assays for Humoral and Cellular Cytotoxicity

Principal Investigator:
Name/Address
Performing
Organization:

Dr. Edwin Matthews
Litton Bionetics
5510 Nicholson Lane
Kensington, Maryland 20795

Contract Number: NO1-CB-53916
Starting Date: 5/09/75
Expiration Date: 5/09/76

Goal: To prepare cultured melanoma cells for immunotherapy vaccine and carry out *in vitro* cytotoxicity tests to measure response of patients to the vaccine.

Approach: 1) Grow human melanoma cells free of contaminants, treat cells with neuraminidase, freeze under sterile conditions, and provide cells as needed for human immunotherapy. 2) Carry out tests for direct cell-mediated cytotoxicity, lymphocyte-dependent antibody and complement-dependent antibody.

Progress: Human melanoma cell lines UCLASM-6, UCLASM-12, and UCLASM-14 have been put into culture and subjected to a variety of quality control tests. As of December 1, 1975, three batches of the pooled allogeneic vaccine had been produced to meet the requirements of the patients entered into the melanoma protocol. During the first few months of the contract period, the contractor has begun to process the humoral and cellular specimens from melanoma patients. Technicians have been trained in the assays they will perform and experiments have been carried out to standardize and make ready the laboratory for analyzing the human blood samples. Assays have been performed on serum and cells from a large number of patient samples.

Significance for Cancer Research (NCP Objective 6 Approach 1)
The melanoma cell line vaccine will be used in a clinical immunotherapy trial.

Project Officer: William D. Terry, M.D., John R. Wunderlich, M.D.
Program: Immunology, Support
Technical Review Group: Ad Hoc Review Committee
Relevance Review Group: DCBD Staff
FY76 Funds: $290,453
Site Visit Date: Waived by memo dated 2/26/75

CONTRACT RESEARCH SUMMARY

Title: Rhesus Monkey Histocompatibility Studies

Principal Investigator: Dr. Bruce Maurer
Name/Address Performing Organization: Litton Bionetics, Inc.
5510 Nicholson Lane
Kensington, Maryland 20795

Contract Number: NO1-CB-64016
Starting Date: 11/01/75 Expiration Date: 10/31/76

Goal: To establish and maintain a colony of pedigreed rhesus monkey families for study of the genetics of transplantation and as an eventual source of donor-recipient pairs for transplant experiments.

Approach: Sera are raised by immunization with skin graft and peripheral blood lymphocytes of selected monkeys previously typed for RhL-A major histocompatibility complex antigens and appropriately matched to related or unrelated donors. Sera are analyzed for serologically defined antigens by titration against known positive and negative monkeys, by microcytotoxicity typing of families and large random unrelated populations and by standard statistical analysis of patterns of reactivity observed. Selected sera are further analyzed for monospecificity by absorption and titration. Monkeys are typed for MLC locus antigens by performing mixed lymphocyte cultures using as stimulators the two available monkeys homozygous at this locus. Breeding is designed to produce RhL-A homozygotes, RhL-A identical siblings, and to enlarge existing families of full siblings.

Progress: Analysis of about 150 alloantisera has resulted in improved definition or new identification of a total of 17 RhL-A alloantigens including 8 in a first series and 9 in a second. (Eleven antigens were previously identified by sera available.) All except 1 antigen is defined by more than 1 serum; in most cases the sera defining a group have very similar frequencies and are very highly correlated ($p<.00001$ in most cases) in X^2 2 x 2 analysis. An antigen possibly belonging to a third linked locus is being evaluated. The MLC homozygotes are being evaluated to establish RhL-A linkage of the MLC locus, frequency of the alleles in the population, linkage disequilibrium with any other RhL-A markers, and significance of the MLC locus for skin transplantation. Breeding has resulted in about 10 live births including the RhL-A homozygote and two new RhL-A identical sibling pairs.

Significance for Cancer Research (NCP Objective 6.1A, Approach 6.6C): Bone marrow transplantation may allow more intensive cancer chemo-or radiotherapy than is presently possible. Transplantation of bone marrow may be important in cancer immunotherapy. Transplantation of other organs may be useful for replacement of tumor-ravaged organs. Such approaches can be studied in outbred animal model if its major histocompatibility complex is sufficiently well analyzed.

Project Officer: J. R. Neefe, Jr., M.D.
Program: Immunology, Support Site Visit: Site Visit Waived by Memo.
Technical Review Group: Ad Hoc Tumor Immunology Review Group
Relevance Review Group: DCBD Contract Program Review Committee
FY76 Funds: $215,887

CONTRACT RESEARCH SUMMARY

Title: Biologic Studies of Solubilized Tumor Antigens

Principal Investigator: Dr. James McCoy
Name/Address Litton Bionetics
Performing Kensington, Maryland
Organization:

Contract Number: NO1-CB-63987
Starting Date: 9/29/75 Expiration Date: 9/28/76

Goal: Conduct immunologic studies of tumors induced by RNA oncogenic viruses and the immune responses they engender.

Approach: Newly induced neoplasms in inbred mice and hamsters are used following transformation in vitro and in vivo by MSV pseudotype viruses. Attempts are being made to evaluate the role played by new "cellular" antigens of the membrane or of viral antigens. Selection of "nonproducer" neoplasms is being attempted. Comparison of the in vivo immune responses to such antigens will be made with in vitro colony inhibition, ^{51}Cr release, proline release, and microcytotoxicity assays. The role of sensitized lymphoid cells and of antibody is being evaluated in the autochthonous host in the induction process of leukemia and of solid tumors using MSV and MSV (MLB).

Progress: Work during this contract period was conducted in close cooperation with the Project Officer and concerned the identification and characterization of TSTA on mineral oil and virus-induced plasmacytomas of mice. Specific emphasis was placed upon studies of the activity of soluble tumor antigens from RNA virus- and DNA virus-induced neoplasms. This part of the work entailed development and standardization of in vitro assays: inhibition of migration of PE cells (an agarose droplet micro method) and lymphocyte stimulation. Specific reactivity was obtained in both systems using soluble and partially purified antigens of spleen cells from $H\text{-}2^a$ mice and from neoplastic cells bearing strong SV40-induced membrane TSTA. Work in progress concerns the possibility of using both systems in assaying various fractions of purified antigens as tools in the purification process.

Significance for Cancer Research (NCP Objective 3 Approach 5)
Knowledge gained through a study of the biology and chemistry of specific tumor antigens on the cell surface of neoplastic cells, transformed by RNA and DNA viruses, will hopefully lead to an understanding of the mechanisms of cancer induction and the prevention and control of neoplasms in man.

Project Officer: Dr. Lloyd W. Law
Program: Immunology, Support Site Visit Date: Site visit requirement
Technical Review Group: Ad Hoc Review Group waived.
Relevance Review Group: DCBD Staff
FY76 Funds: $215,542

CONTRACT RESEARCH SUMMARY

Title: Measurement of Immunological Reactivity to Human Cancer

Principal Investigator: Dr. James McCoy
Name/Address Litton Bionetics, Inc.
Performing 5510 Nicholson Lane
Organization: Kensington, Maryland 20795

Contract Number: N01-CB-63975
Starting Date: 11/01/75 Expiration Date: 8/31/77

Goal: To study the immune responses of cancer patients to tumor associated antigens, and to correlate results of different assays.

Approach: Immunological tests have been set up to evaluate and monitor the immune competence of patients and their response to tumor associated antigens, including: lymphocyte stimulation by mitogens, recall antigens, rosette assays for enumeration of T and B cells; leukocyte migration inhibition assays; delayed hypersensitivity skin tests; and lymphocyte cytotoxicity assays against tumor cells. Several cancers and matched control patients are studied.

Progress: Most of the assays were standardized and the emphasis was placed on serial testing of patients. Patients with carcinoma of the lung, breast, malignant melanoma, and Ewing's sarcoma were studied. The majority of patients had decreased percentages of T cells by the 29° rosette assay. In carcinoma of the lung, depressed or falling rosette values were associated with poor prognosis and recurrent disease, about three months prior to detection of recurrence. In studies of breast cancer, administration of Corynebacterium parvum caused an elevation in depressed rosette levels, while the 29° rosette assay was considerably more sensitive in detecting depressed levels in cancer patients, as compared to the assays performed at 4°. Experience was gained with a micromodification of the migration inhibition assay: the microdroplet agarose assay of Harrington. Using tuberculin as a model antigen, some skin test positive individuals were shown to be reactive at nanogram concentrations of PPD. The general immunologic competence of cancer patients was also monitored in the lymphocyte stimulation assay with mitogens and with mixed leukocyte cultures. Autologous proliferative responses of patients with lung and breast cancer to autologous tumor cells and tumor cell extracts showed that approximately 50% of the patients had positive reactions, apparently directed against tumor associated antigens. In cytoxicity assays, the major emphasis has been placed on understanding of normal cytotoxic reactivity, demonstrating that the effector cells are lymphocytes with receptors for immunoglobulin, most of them lacking complement receptors and forming no rosettes with sheep erythrocytes under usual conditions except with neuraminidase treated sheep erythrocytes.

Significance for Cancer Research (NCP Objective 5 Approach 4) Comparison between severl assays of cell-mediated immunity in tumor patients and the usefulness in immunodiagnosis of cancer and in following the clinical course of patients will be provided.

Project Officer: Ronald B. Herberman, M.D.
Program: Immunology, Support Site Visit Date: Requirement waived
Technical Review Group: Ad Hoc Tumor Immunology Review Group
Relevance Review Group: DCBD Contract Program Review Committee
FY76 Funds: $509,294

CONTRACT RESEARCH SUMMARY

Title: Induction, Transplantation and Preservation of Plasma Cell Tumors in Mice

Principal Investigator: Dr. Kyle Sibinovic
Name/Address Litton Bionetics
Performing Bethesda, Maryland 20014
Organization:

Contract Number: NO1-CB-92142
Starting Date: 6/23/69 Expiration Date: 1/31/77

Goal: Induction, transplantation and preservation of plasma cell tumors in mice.

Approach: Contractor preserves and transplants plasmacytomas in a liquid nitrogen bank and provides and ships these tumors on request to the NCI, and other investigators working with mouse plasmacytomas. In addition the contractor induces new plasmacytomas. Series of plasmacytomas producing antigen-binding myeloma proteins for the same hapten are being collected. Myeloma proteins from sources other than BALB/c are also being collected. Contractor assists project officer in the study of the mechanism of priming and tumor specific immunity to plasmacytomas. Contractor provides ascites containing myeloma proteins to NCI.

Progress: The contractor's level of effort and willingness to improve have been excellent. Many requests were received for tumors from the US (NIH and non-NIH) and foreign investigators. The contractor processed these tumors for distribution. The tumor bank has been expanded to include thymus-derived lymphocytic neoplasms, non-thymus derived lymphocytic neoplasms (eg. B-cell lymphomas induced by Avelson virus), new mastocytomas and reticulum cell sarcomas. The contractor is developing its capability to perform several additional characterizations on tumors in the bank, and it has performed and completed a series of plasma-cytoma induction experiments. Viability control experiments have been initiated and it is hoped that these will lead to improved methods of freezing and recovery of tumors. The contractor has greatly increased its proficiency in quality control evaluation, and the data retrieval system has been computerized. The contract has housed a colony of 5,800 mice and delivered them on request to the Project Officer. In addition, the contractor has bred and in maintaining several mouse lines which are used in studies of transplantation antigens.

Significance for Cancer Research (NCP Objectives 6,4 Approaches 1,4)
Supplies essential biological material for investigators studying the biology of neoplastic plasma cells, tumor immunology, the genetics of immunoglobulins, and immunoglobulin synthesis.

Project Officer: Michael Potter, M.D., David H. Sachs, M.D.
Program: Immunology, Support Site Visit Date: None (The Project
Technical Review Group: Ad Hoc Review Group Officer visits the site)
Relevance Review Group: DCBD Staff
FY76 Funds $40,320 (Supplement) $310,474

CONTRACT RESEARCH SUMMARY

Title: Hematoxylin Substitutes for Diagnostic Staining of Tissues and Exfoliated Cells

Principal Investigator: Ralph Lillie
Name/Address Performing Organization: Louisiana State Univ. Medical Center
New Orleans, Louisiana 70112
Department of Pathology, LSU Medical Center

Contract Number: NO1-CB-43912
Starting Date: 1/10/74 Expiration Date: 1/10/79

Goal: Develop dyes that will adequately replace the conventional hematoxylin now used for routine staining of histological and cytological preparations.

Approach: Synthesize and discover by other means potential dyes for this purpose. Test such dyes and review results.

Progress: Several reasonable substitutes are being tested exhaustively for compatibility with present systems. These include Phenocyamin TC and other orcein type preparations.

Significance for Cancer Research: Innovative staining techniques for the examination of tissues are essential to better diagnostic methods.

Project Officer: Mearl F. Stanton, M.D.
Program: General Contract Site Visit Date: Deferred
Technical Review Group: Ad Hoc Technical Review Group on Histolog. Diagnosis
Relevance Review Group: DCBD Contract Review Board
FY76 Funds: $46,362

CONTRACT RESEARCH SUMMARY

Title: National Cancer Institute Immunodiagnostic Reference Center

Principal Investigator: Kenneth Kortright, Ph.D.
Name/Address Performing Organization: Meloy Laboratories, Inc.
Springfield, Virginia 22151

Contract Number: NO1-CB-63976
Starting Date: 7/11/75 Expiration Date: 7/10/76

Goal: To use quantitative and qualitative immunochemical techniques for improved diagnosis of cancer and to evaluate humoral immune responsiveness of patients with cancer.
Approach: Sensitive double antibody radioimmunoassays are being used to quantitate alphafetoprotein (AFP) and human chronic gonadotropin (hCG) in serum and other biological fluids. These assays are being evaluated as techniques for diagnosis of cancer and to define the response to therapy. Appropriate reagents for these assays are being prepared and evaluated. In addition alphafetoprotein is being purified for use in metabolic turnover studies and in studies to define the immunosuppressive properties of alphafetaprotein. In addition the immunodiagnostic center performs tests for humoral competence of patients with cancer or with immunodeficiency diseases that have a high incidence of associated neoplasms. Finally the immunodiagnostic center serves as a distribution outlet for provision of reference material for human serum immunoglobulin quantitation to qualified laboratories and in cooperation with the World Health Organization prepares and distributes international immunoglobulin reference serums. The Immunoglobulin Reference Center will also serve as a distribution point for carcino-fetal standard preparations when they are available. Finally, the Reference Center serves as a focus for the education of investigators and their technicians in the techniques and interpretations of various immunodiagnostic procedures.
Progress: AFP has been shown to be elevated in a very high percentage of patients with cancer of the liver and testes and a lower percentage of patients with entodermally derived tumors but in virtually no patients with benign nonhepatic disorders. The AFP and hCG assays have been of great value for following the effectiveness of therapy for testicular germinal neoplasms providing an excellent marker for residual tumor cells. In many cases marker elevations are the sole evidence of the residual cancer. However, there have been no false positive determinations. All patients with an elevated marker have a clinically evident recurrence within a year.

Significance for Cancer Research (NCP Objective 5 Approach 4)
A center with the capability of performing multiple immunodiagnostic tests will help to rapidly establish the usefulness of these tests in the diagnosis of cancer or as methods for monitoring patients during and after therapy.

Project Officer: Thomas A. Waldman, M.D., M. Blaese, M.D., Robert McIntire, M.D.
Program: Immunology, Support Site Visit Date: Project Officer visits the site
Technical Review Group: Ad Hoc Review Group
Relevance Review Group: DCBD Staff
FY76 Funds $222,118

CONTRACT RESEARCH SUMMARY

Title: Immunodiagnostic Testing, Antibody Measurement and Fractionation of Serum and Urine

Principal Investigator: Roy Woods, Ph.D.
Name/Address Performing Organization: Meloy Laboratories, Inc.
Springfield, Virginia 22151

Contract Number: NO1-CB-33875
Starting Date: 11/26/65 Expiration Date: 7/10/75

Goal: To use quantitative and qualitative immunochemical techniques for improved diagnosis of cancer and to evaluate humoral immune responsiveness of patients with cancer.

Approach: Qualitative and quantitative immunochemical techniques are used for the diagnosis of multiple myeloma, Waldenstrom's macroglobulinemia, agammaglobulinemia, and other diseases with immunoglobulin disorders. In addition, these same techniques are used to evaluate carcinoembryonic antigen and α-fetoprotein in the serum of patients with a variety of malignancies. Studies are carried out to classify the abnormal proteins of the abnormality diseases and to study the α-fetoprotein and carcinoembryonic antigens in relation to diagnosis, course of disease, and response to therapy. Appropriate reagents for the immunoglobulin and carcino-fetal antigen detections are prepared and evaluated. In addition, the Immunoglobulin Reference Center serves as a distribution outlet for provision of reference material for human serum immunoglobulin quantitation to qualified laboratories and in cooperation with the World Health Organization prepares and distributes international immunoglobulin reference serums. The Immunoglobulin Reference Center will also serve as a distribution point for carcino-fetal standard preparations when they are available. Finally, the Reference Center serves as a focus for the education of investigators and their technicians in the techniques and interpretations of various immunodiagnostic procedures.

Progress: CEA assays have been used to follow patients with metastatic disease during drug therapy. Preliminary data indicate that CEA fluctuates with, but does not precede, other measures of tumor load in patients with advanced cancer. Alphafetoprotein and human chorionic gonadotrophin levels are also being determined on serums from patients with cancer.

Significance for Cancer Research (NCP Objective 5 Approach 4)
A center with the capability of performing multiple immunodiagnostic tests will help to rapidly establish the usefulness of these tests in the diagnosis of cancer or as methods for monitoring patients during and after therapy.

Project Officer: W. D. Terry, M.D., M. Blaese, M.D., T. A. Waldmann, M.D.
Program: Immunodiagnosis Site Visit Date: 11/19/73
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY75 Funds: $174,085

CONTRACT RESEARCH SUMMARY

Title: Preparation of Reagent Antisera and Antigens

Principal Investigator:
Name/Address
Performing
Organization:

Dr. Howard M. Grey
National Jewish Hospital and Research Center
3800 East Colfax
Denver, Colorado 90206

Contract Number: NO1-CB-53912
Starting Date: 5/16/75 Expiration Date: 5/15/76

Goal: Preparation of monospecific fluorescent-isomer conjugated IgG $F(ab')_2$ antibodies to all heavy and light chain classes of human and mouse immunoglobulins and human B and T lymphocytes.

Approach: All classes of human and mouse heavy and light chains will be purified by immunochemical techniques. These will be used to immunize animals and will be conjugated to Sepharose beads. The resulting antisera will be absorbed with appropriate conjugated beads to render them monospecific. The IgG fractions of such antisera will be isolated, enzyme digested to make $F(ab')_2$ fragments, and conjugated to fluorescent isomers. A variety of cell types will be used for both immunization and absorption to make human B and T cell specific antisera. The antibodies will then be further processed as for the anti-Ig reagents.

Progress: (New Contract) Antisera to all human Ig classes and subclasses have been raised in rabbits, the IgG fractions obtained and appropriately absorbed. Enzyme digestions and fluorescent isomer conjugations are being performed at this time. Mouse Ig classes and subclasses are being prepared and when this is complete, immunizations and sepharose bead conjugations will proceed. Rabbits are presently being immunized with monkey thymocytes and the resultant sera will be tested for antibody activity on human lymphocytes.

Significance for Cancer Research (NCP Objective 3 Approach 5)
To provide specialized antisera required for cell surface studies important in the Baseline Information Flow of the Tumor Immunology Program.

Project Officer: Howard B. Dickler, M.D., Warren Strober, M.D.
Program: Immunology, Support Site Visit Date: 3/4/76
Technical Review Group: Ad Hoc Review Committee
Relevance Review Group: DCBD Staff
FY76 Funds: $71,811

CONTRACT RESEARCH SUMMARY

Title: Production of Human Lymphoid Cells in Culture

Principal Investigator: Dr. Sami Mayyasi
Name/Address Performing Organization: Pfizer, Inc.
199 Maywood Avenue
Maywood, New Jersey 07607

Contract Number: N01-CB-12078
Starting Date: 1/01/71 Expiration Date: 2/01/76

Goal: To grow and produce large quantities of human lymphoid cells in long-term tissue culture and to grow the Raji cell line in human serum for immunization of acute leukemia patients.

Approach: Several human lymphoid tissue culture cell lines which bear different HL-A alloantigens are produced in large quantities and are isolated, concentrated, and shipped to the Project Officer for subsequent use in the isolation and characterization of the HL-A alloantigens. One of the cell lines, Raji, is being grown in quantities sufficient to continue an immunization program in acute leukemia in humans. This cell line, unlike the other cell lines, is grown in normal human serum to eliminate the possibility of immunization with the fetal bovine serum foreign protein.

Progress: During the last contract period, 7316 grams of lymphoblastoid cells were supplied to the Project Officer. These included 2575 grams of murine cells. 137 grams of the RAJI cell line was produced. Approximately 60 grams of cells were labeled with titrated amino acids or 35 S methionine.

Significance for Cancer Research (NCP Objective 3 Approach 5)
Provides prototype technique for the isolation of tumor specific or tumor associated antigens. Raji cell line has been used for immunotherapy in acute leukemia.

Project Officer: Dean L. Mann, M.D.
Program: Immunology, Support Site Visit Date: June 1972
Technical Review Group: Ad Hoc Tumor Immunology Review Group
Relevance Review Group: Ad Hoc DCBD Contract Program Review Committee
FY76 Funds: $21,619

CONTRACT RESEARCH SUMMARY

Title: Small Animal Holding Facility

Principal Investigator: Andrew S. Tegeris
Name/Address Performing Organization: Pharmacopathics Research Laboratories, Inc.
9505 N. Washington Blvd.
Laurel, Maryland 20810

Contract Number: NO1-CB-33887
Starting Date: 7/01/73 Expiration Date: 6/30/78

Goal: To maintain and observe animals on experiments designed to develop the means to reduce the effectiveness of external agents for producing cancer with a variety of types of materials, particularly mineral fibers and tobafco products.

Approach: Improve animal models of human cancer and evaluate effects of combined factors by assessing the response of the pleura of the rat to fibers and other forms of minerals that could be expected to reach this site in man.

Progress: We are currently testing carcinogenic response to many types of durable fibers in the pleura of the rat. Results suggest that fiber size is critical to carcinogenesis by asbestos and other fibers.

Significance for Cancer Research: Animal models of human cancer are essential to identifying causative agents, mechanisms of action and therapeutic trials.

Project Officer: Mearl F. Stanton, M.D.
Program: General Contract Site Visit Date: Monthly
Technical Review Group: Ad Hoc Relevance Review Group
Relevance Review Group: DCBD Contract Review Board
FY76 Funds: $125,605

CONTRACT RESEARCH SUMMARY

Title: A Study of Phylogenetic Aspects of Neoplasia

Principal Investigator: John C. Harshbarger, Ph.D.
Name/Address The Smithsonian Institution
Performing Washington, DC 20560
Organization: Registry of Tumors in Lower Animals

Contract Number: NO1-CB-33874
Starting Date: 7/01/73 Expiration Date: 6/29/76

Goal: To collect, examine, classify, and preserve neoplasms in cold-blooded vertebrate and invertebrate animals, and to study experimentally the development of tumors in lower animals.

Approach: The Principal Investigator directs the operation of a Registry of Tumors in Lower Animals. Specimens are acquired from personal field investigations or through submittal by other investigators. The specimens are examined grossly, histologically, and in some cases by electron microscopy. Diagnoses are established and the specimens are described. The world literature on tumors in lower animals is collected. Field investigations and experimental inductions of tumors in lower animals are carried out. Publications of the findings are made. The Registry also serves in a consulting capacity to other agencies concerned with diseases in lower animals, such as the Environmental Protection Agency, the U.S. Bureau of Fisheries (Commerce Dept.), and the Environmental Health Center.

Progress: Specimen accessions during the past year numbered more than 300, bringing the total collection to 1,400 specimens accumulated during a 10 year period. About half the total number of specimens are neoplasms. The majority are in teleost fishes, reptiles, and amphibians, with relatively few in two invertebrate phyla, the molluscs and arthropods. Outstanding accessions during 1975-76 included a series of 47 tumors described in hagfish by a Swedish collector, and a collection of 102 examples of gonadal tumors in soft-shell clams. Both groups of neoplasms are under investigation to determine whether they may be related to environmental carcinogens. Data continue to indicate that certain species are prone to develop neoplasms of a particular type characteristic for that species. In some instances there is also correlation between geographic location and tumor proneness within a given species.

Significance for Cancer Research: Field studies and anatomical studies indicate environmental carcinogens that may be of importance in human cancer epidemiology or may be useful in designing analytical experiments to determine mechanisms of tumorigenesis.

Project Officer: Clyde J. Dawe, M.D.
Program: Cancer Biology Support Site Visit Date: 1/28/76
Technical Review Group:
Relevance Review Group: DCBD Contract Review Board
FY76 Funds: $119,717